The Giant Liver Fluke: A Review, Intermediate Host Habitat, and Infection in a White-tailed Deer Population in Minnesota

A Thesis
SUBMITTED TO THE FACULTY OF
UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

Dr. Ronald A. Moen

May 2016

Acknowledgements

I am grateful for the assistance of Dr. Ron Moen in the many readings of this manuscript and support throughout my thesis work.

Dr. John Pastor and Dr. Bert Stromberg, for support and advice.

The Biology Department of the University of Minnesota – Duluth for graduate teaching assistantship funding and support.

The Environmental and Natural Resources Trust Fund; The Clean Water, Land, and Legacy Amendment; and the Minnesota Zoo for financial support.

Swenson College of Science and Engineering and the *Alces* Newcomer's travel grant for travel funding to present this work.

The Arrowhead Bowhunters' Alliance for providing deer livers.

My wife, Heather Vannatta, for reading many papers, proposals, and manuscripts.

Dedication

I dedicate this work to my family and friends who have helped make me who I am. I would especially like to mention Heather and Evelyn for being a constant source of support. Also, my grandmothers, Momo and Grandma Halbe. Thank you.

Table of Contents

List of Tables

List of Figures

Chapter 1:

Giant Liver Fluke in North American Cervids: Just a Fluke?

Summary

Fascioloides magna is a potential contributing factor to moose mortality across North America. Although the effect of this parasite on moose populations is debated, there is little evidence that *F. magna* infection alone will cause mortality. However, *F. magna* prevalence is increasing, and the additional parasite burden may be important in combination with other factors. *F. magna* has four primary life cycle stages with different developmental requirements that limit the habitats in which cervids can become infected. The least understood aspect of *F. magna* infection risk to cervids is the ecology of its snail intermediate hosts. Which snails are most important for *F. magna* transmission is not fully known. The habitat requirements and fine-scale distribution of these snails is also not well understood. However, snail hosts must overlap with cervid feeding or drinking in aquatic habitats for transmission of *F. magna* to occur. Moose feed on aquatic vegetation, but aquatic feeding in white-tailed deer and elk, the primary hosts of *F. magna*, is not well understood. Differences in aquatic feeding habits of moose and deer may generate different infection risks. Habitat use, population density, and age structure of sympatric deer and elk likely contribute to infection risk for moose. We suggest future research should focus on *F. magna* as a contributing factor, and not the direct cause of mortality in cervids. Examining the energetic cost and fitness losses associated with *F. magna* infection, followed by a comprehensive risk assessment using known habitat requirements of *F. magna*, lymnaeid snails, and cervid hosts would elucidate the risks and impacts of *F. magna* in North America.

Introduction

Fascioloides magna, the giant liver fluke, is a trematode parasite that infects white-tailed deer (*Odocoileus virginianus*), elk (*Cervus canadensis*), and caribou (*Rangifer tarandus*) across North America (Fig.1; Pybus 2001). Moose (*Alces alces*) and other cervids often become infected with *F. magna* where their geographic range overlaps with natural fluke hosts (Karns 1972, Lankester 1974, Pybus 2001). As their name suggests, giant liver flukes take up residence in fibrous capsules within cervid livers (Fig. 2). Although liver damage associated with *F. magna* infection is visually striking, most white-tailed deer sustain little loss of function in liver tissues (Presidente et al. 1980).

The damage which does occur during the initial stages of infection in deer does not substantially decrease fitness (Pursglove et al. 1977, Presidente et al. 1980, Mulvey and Aho 1993, Mulvey et al. 1994). In contrast, moose, a dead-end host, display a strong immune response, which some authors have suggested may contribute substantially to moose mortality (Murray et al. 2006). This heightened immune response is thought to contribute to thickening of fluke capsule walls, the inability of flukes to release eggs from capsules, and the eventual death of the parasite (Lankester 1974). Remnant capsules are often filled with dead flukes and other waste, which may impair liver function (Pybus 2001). In the migratory phase of their life cycle, immature *F. magna* appear to migrate extensively in moose, sometimes damaging >50% of the liver tissue, which may lead to secondary infections (Karns 1972, Lankester 1974, Pybus 2001).

Whether damage from *F. magna* infection increases mortality in moose is debated, but recent declines in moose populations at the southern edge of their range

emphasize the need for further examination of *F. magna* as a mortality factor. Murray et al. (2006) concluded ~20% of moose mortality was probable *F. magna* infection. However, this source of mortality was partly a classification by exclusion. If no other signs of mortality were present and moose liver tissue contained an abundance of fluke damage, these individuals were classified as probable fluke mortalities. Lankester (2010) notes that the *F. magna* infection prevalence between 1972 (87%; Karns 1972) and the beginning of Murray et al.'s study in 1995 (89%) did not change even though the moose population increased over this period.

Additionally, captive moose experimentally infected with *F. magna* showed no outward symptoms of infection (Lankester and Foreyt 2011). Although this study was done on only three individuals raised in captivity with adequate food, minimal stress, and no predation pressure, marked symptoms would be expected if *F. magna* was a severe pathogen of moose. When a moose is free-ranging, the energy expenditure involved in tissue repair and immune response to infection could create an additive energetic cost in concert with severe malnutrition, predation pressure, and other pathogens. The biotic and abiotic factors dictating infection risk in moose remain an important issue for research and management.

In this review, we summarize the current state of knowledge regarding *F. magna*'s interactions with the abiotic environment, lymnaeid snail intermediate hosts and their taxonomy, known cervid hosts of *F. magna,* their aquatic foraging behaviors, historic increases in *F. magna* prevalence, and how giant liver flukes may relate directly and indirectly to host mortality.

Fascioloides magna

Fascioloides magna completes its life cycle using a snail, a plant, and a cervid sequentially as hosts (Swales 1935, Pybus 2001). First, eggs released in deer pellets develop into miracidia, ~0.2 mm ciliated larvae (Fig. 3). The miracidium penetrates the foot of an aquatic lymnaeid snail, then develops into a sporocyst, which gives rise to many mother rediae. Mother rediae give rise to many daughter rediae, which release free-swimming cercariae from the snail. Sporocysts and rediae are largely nondescript, appearing as white sacks filled with the next life cycle stage (sporocyst → mother rediae → daughter rediae → cercariae). The sperm-like cercariae encyst as ~0.25 mm diameter, helmet-shaped metacercariae on aquatic vegetation where they are eventually ingested by a cervid. Metacercariae excyst and penetrate the intestine as juvenile flukes. Juvenile flukes migrate along the body wall until reaching the liver (Sukhdeo and Sukhdeo 2002). Once in the liver, immature flukes migrate extensively until they find one or more other flukes (Foreyt et al. 1977). This migration is visible as black tracks, scarring, and hemorrhaging. Paired flukes settle in the liver and stimulate the host to form a collagen-based capsule in which the flukes live (Wolf-Spengler and Isseroff 1983).

Eggs and miracidia: The eggs of *F. magna* are sensitive to temperature and humidity (Pybus 2001). The threshold temperature for egg development is likely 10 to 12ºC, although development is slow at these temperatures (Campbell 1961, Pybus 2001). The rate of egg development increases as temperature increases. A 10ºC increase can nearly double the development rate of miracidia (Campbell 1961).

The developmental threshold of 10°C appears high for sustained *F. magna* populations in northern Canada, but microsite conditions may be warmer. An *F. magna* population is present in caribou near Ungava bay at the Quebec-Newfoundland border (Lankester and Luttich 1988, Pybus 2001). The success of *F. magna* in northern Canada suggests that shallow water habitat, which warms quickly in early summer and can remain above this threshold for ~30 days is the most likely location for *F. magna* survival and subsequent snail and cervid infection. These abiotic conditions are necessary for complete development of *F. magna* eggs. Shallow waters, such as ephemeral ponds and wetlands, may be infection sources at higher latitudes.

The eggs of *F. magna* are resistant to cold temperatures (Swales 1935, Campbell 1961, Friedl 1961). Even after eggs are stored for six to nine months at 5°C, they still maintain high hatching rates (Swales 1935). Eggs stored at 5°C for over two years still had successful miracidial emergence (Friedl 1961). This suggests eggs dropped over winter and insulated from temperature extremes may retain the ability to mature in spring.

Humidity also plays an important role in egg development for *F. magna* and closely related species, such as *Fasciola hepatica* (Swales 1935, Rowcliffe and Ollerenshaw 1960, Pybus 2001). Below 50% relative humidity eggs became desiccated and would not produce viable miracidia (Swales 1935). A critical moisture film may evaporate from the egg surface when relative humidity is less than 50% (Rowcliffe and Ollerenshaw 1960). This threshold may limit the distribution of *F. magna* in dry regions

such as the Great Plains (Pybus 2001, Maskey 2011) and likely dictates which habitat types are of greatest risk for infection.

Light and circadian rhythms have also been indicated as potential factors in trematode egg hatching (Friedl 1961, Behrens and Nollen 1993). Miracidia of the trematode *Echinostoma caproni* did not hatch in the absence of light (Behrens and Nollen 1993). Parasite eggs buried in lake sediments or otherwise away from light may never emerge to infect a snail unless the substrate is disturbed. However, mass hatching of eggs can be stimulated by a major change in the aquatic environment such as, dissolved oxygen, temperature, or pH (Swales 1935, Campbell 1961). This suggests that very broad habitat requirements are suitable for miracidial hatching and that some form of disturbance such as rain, a snail which is disturbing the sediment, or an increase in dissolved oxygen with photosynthetic activity could stimulate hatching.

Temperature and humidity affect maturation of the egg and survival of a hatched miracidia, but the miracidial stage is also limited by energy stores. Miracidia are free-living larval forms and do not feed. This restricts miracidial dispersal based on stored energy and proximity of a suitable snail host. During this time, various phototactic and chemotactic behaviors allow a miracidia to locate hosts, but stored energy may only last between eight and 48 hours (Campbell 1961, Friedl 1961). The dispersal time would vary with the proximity of snails, which induce swimming behaviors and energy expenditure in miracidia (Swales 1935, Campbell and Todd 1955a).

Sporocysts and rediae: Upon penetration of a snail, a miracidium develops to a single sporocyst. From a sporocyst many mother rediae arise, which release many daughter rediae which release many cercariae. Pybus (2001) estimated that a single miracidium could potentially give rise to ~1200 cercariae. Field and laboratory experiments suggest the actual number of cercariae released per infected snail ranges from ~10-200 depending on species (Foreyt and Todd 1978, Vignoles et al. 2006). These reproductive potentials likely compensate for a seeming infection paradox in which >50% of definitive hosts may be infected with *F. magna*, but only 0.16% to 2% of intermediate hosts become infected (Erhardova-Kotrla 1971, Laursen and Stromberg 1993, Vanderwaal et al. 2014). Asexual reproductive output remains possible as long as the snail host is alive. Sporocysts and rediae can overwinter in snail hosts and continue reproduction when conditions become favorable (Griffiths 1959, Erhardova-Kotrla 1971).

Cercariae and metacercariae: Factors affecting cercarial emergence and metacercarial encystment in species similar to *F. magna* have been extensively reviewed by Morley (2015). Therefore, we only review the most pertinent conceptual and species specific information here.

Cercariae face much the same adversities as miracidia. They must find a suitable location for encystment before desiccation or before energy stores are depleted. In some cases, this larval stage may be susceptible to predation (Johnson et al. 2010). When seeking a location for encystment, cercariae swim past vegetation in the immediate vicinity (Dreyfuss et al. 2004) and encyst on green vegetation (Hodasi 1972). Both these

adaptations increase the probability of ingestion by a definitive host by dispersing propagules onto quality aquatic forage.

The encysted metacercariae survive best under humid conditions. Metacercariae have survived for over 100 days at room temperature and over 200 days in refrigerated conditions with adequate humidity (Griffiths and Christensen 1972). Under the right conditions, metacercarial abundance could increase throughout the summer months, making visits to aquatic habitat progressively more hazardous for cervids in late summer and possibly into fall and winter. Metacercariae stored at 3-5ºC for 393 days remained infective (Griffiths and Christensen 1974), suggesting that metacercariae in unfrozen water could remain infective during winter. Although temperatures this low would prevent development of the egg and intra-molluscan stages, metacercariae may survive. Since moose have been documented using mineral licks year-round (Rea et al. 2013), infection during the winter months warrants consideration. In a study of seasonal transmission, Craig and Bell (1978) showed that *F. magna* transmission in Texas peaked in January when the average temperature was 14ºC. It is not unreasonable to speculate that the low temperatures in January could have prolonged metacercarial viability and parasite transmission. Nevertheless, the relative tradeoffs between long term metacercarial survival and future adult parasite fitness are unknown (Morley 2015).

Additionally, cercariae likely do not always emerge in close proximity to suitable aquatic vegetation. In these cases, and possibly even in the presence of suitable vegetation, some cercariae may encyst as free-floating metacercariae at the water's surface (Morley 2015). Little research has been done on free-floating metacercariae, but

in human fascioliasis some patients have no other reported routes of infection except for ingestion of free-floating metacercariae while drinking water (Mas-Coma 2004, Morley 2015). It is also possible that cervids could become infected with *F. magna* by drinking water or by eating soils at a mineral lick.

Adult: Once in the stable definitive host environment, external abiotic factors such as temperature and humidity are unlikely to affect the health or reproductive output of an adult fluke. In elk, adult giant liver flukes release between 4,000 and 7,000 eggs per day per fluke (Swales 1935, Pybus 2001). However, this rate of egg production is unlikely to hold in all cases. Egg release is not proportional to infection intensity and assumptions of infection intensity based on eggs in feces should be approached carefully (Boray 1969, Blazek et al. 1972). Additionally, only 80% of eggs are likely viable (Rowcliffe and Ollerenshaw 1960). Regardless of variation in egg viability, a single infected white-tailed deer or elk within a moose's home range could pose a serious infection risk.

Lymnaeid snails

The taxonomy, ecology, relative importance, and North American geographic range of specific lymnaeid snail hosts remains a poorly understood and much debated topic. We focus our discussion on the ecology and geographic range of known *F. magna* host snails using the most current taxonomic information (Table 1).

The correct taxonomy of lymnaeid snails is still debated. Morphology-based taxonomy greatly confused the study of lymnaeid snails by creating several invalid species classifications (Baker 1911a, Hubendick 1951, Dillon Jr. et al. 2013). The varied

growth patterns of lymnaeid snails in different environments is likely to blame for these taxonomic errors (Hubendick 1951). More recently, genetic analyses have revealed that almost all North American lymnaeid species are in a single clade and that lymnaeids will soon fall into the genera *Catascopium* and *Hinkleyia* to reflect North America's shared ancestry (Correa et al. 2010). Snails in the *Stagnicola* genus (*Lymnaea elodes* and *L. catascopium* from Table 1) may actually represent a single species of the new genus *Catascopium*. However, for ease of interpretation these potential conspecifics will be treated as separate species here. As a final taxonomic note, there is likely no group of lymnaeids as taxonomically confused as the *Fossaria* genus (Stewart and Dillon Jr. 2004). In light of this confusion, representatives of this group will be referenced as *Lymnaea* in this paper and include *L. humilis, L. bulimoides, L. dalli, L. cubensis,* and several others. We will consider all these species under the classification of *Fossaria* spp., except for the circum-Caribbean snail, *L. cubensis*, which has been recognized as distinctive for some time (Baker 1911a, Hubendick 1951, Dillon Jr. et al. 2013).

Three lymnaeid snail intermediate hosts can be naturally infected with *F. magna*: *Fossaria* spp., *Lymnaea caperata,* and *L. elodes* (Table 1; Swales 1935, Laursen and Stromberg 1993). However, when *F. magna*'s geographic range is overlaid with those of the intermediate snail hosts, no combination of known, natural intermediate hosts fully explains *F. magna*'s geographic distribution (Fig. 4A, B). Of primary interest are the *F. magna* populations in Labrador, Canada and Florida, USA. Although natural infections in *L. elodes* could explain *F. magna*'s presence in Labrador, it is also possible that yet undocumented *Fossaria* spp. snails are present in this area. In Florida, it is most likely

that *L. cubensis* is an undocumented natural host. *L. cubensis* is a host of the closely related liver fluke, *Fasciola hepatica,* in Latin America (Cruz-reyes and Malek 1987). Not only is *L. cubensis* closely related to other *Fossaria* spp. snails (Correa et al. 2010), but it shares similar habitat characteristics (Table 2). *Fossaria* spp. and *L. caperata* snails known as natural hosts are amphibious snails common to vernal pools and ephemeral water habitats. *L. caperata, L. elodes,* and *L. catascopium* are very closely related, yet natural infection in *L. catascopium* has not been documented. Thus, the large lakes and rivers inhabited by *L. catascopium* are likely poor habitat for *F. magna* miracidia and stagnant waters of vernal pools and ponds appear more important for miracidial transmission to intermediate hosts. *L. elodes* has been found naturally infected and is often found in larger lakes, but it is likely the smaller ponds and marshes this species also inhabits where infection occurs.

The habitat preferences of lymnaeid snails are most pertinent as they relate to initial exposure to giant liver fluke miracidia and the eventual encystment location for metacercariae. In order for successful infection of a definitive host, intermediate host snails must be present in a water source frequented by a cervid host. This relationship links snail-habitat associations and cervid aquatic feeding behavior to giant liver fluke infection risk. In the closely related fluke, *Fasciola hepatica*, researchers have used snail-plant associations along with other factors to predict where snails, and thus infection risk, are more common (Rondelaud et al. 2011). The presence of *Fossaria* spp. snails in vernal pools and ephemeral wetlands points to emergent aquatic vegetation as the primary

source of giant liver fluke infection. As water levels drop, metacercariae would be above the water line and exposed to foraging deer (Swales 1935).

Aquatic vegetation

Moose are well documented aquatic feeders (DeVos 1958, Hennings 1977, Belovsky and Jordan 1981, Fraser et al. 1982, 1984), but aquatic feeding in white-tailed deer and other cervids is not well understood (Skinner and Telfer 1974, Hennings 1977, Ceacero et al. 2014). Most literature suggests that sodium requirements drive the aquatic feeding habits of moose (Belovsky and Jordan 1981, Fraser et al. 1982, Ceacero et al. 2014). Although this is possible, recent evidence suggests that protein content and seasonal nutrient requirements are also important drivers (Ceacero et al. 2014). Seasonal variation in aquatic habitat use by moose is common (de Vos 1958, Belovsky and Jordan 1981, Fraser et al. 1984, Moen et al. unpublished data). Most observations show peak aquatic use in late June or early July, slowly decreasing through late September. A similar seasonality is apparent in aquatic feeding by white-tailed deer (Hennings 1977). This seasonal trend correlates with metacercarial production. Once above the 10ºC egg development threshold, new metacercariae would be expected in ~75 days (Pybus 2001). Assuming a 10ºC threshold is reached in shallow aquatic habitats in early May, current year's metacercariae should begin to appear in mid-July when deer and moose feed on aquatic plants.

In light of knowledge regarding snail host habitat, early and late season feeding near vernal pools and muddy lake and stream edges is more likely to lead to giant liver

fluke infection. As temperatures increase in early spring, snails that carry infections over winter are likely to generate a pulse of metacercariae. Muddy water edges and vernal pools often contain the first green vegetation in spring (Frank and Mcnaughton 1993, Robins and Vollmar 2002). Moose and other cervids could come to the water's edge at this time and feed on the first green vegetation of the year, becoming infected. This general trend could also hold true in fall, as vegetation near the water's edge remains green later into the season. Additionally, consumption of leaf litter in fall could present an infection risk (Jenkins and Starkey 1993). These spring and fall feeding habitats match known peaks in metacercarial encystment (Lepitzki 1998).

Although spatial and temporal components of aquatic feeding are similar in white-tailed deer and moose (Hennings 1977), these species show differing preference for aquatic plants. Aquatic feeding by moose has been extensively reviewed by Morris (2014). Moose have a strong preference for submerged aquatic vegetation (Table 3; Fraser et al. 1984). In the few studies of aquatic vegetation consumption by white-tailed deer, deer consumed similar numbers of emergent and floating/submergent plant species (Table 4; Skinner and Telfer 1974, Hennings 1977, Watkins and Ullrey 1983). Whether the different foraging behaviors of moose and deer lead to different infection risks is unknown. However, it seems most likely that moose are infected when feeding in vernal pools and other areas as outlined above. Assuming foraging on submergent vegetation was an infection risk, moose would most likely become infected feeding in the littoral zone at < 1 m water depth or early in the year feeding on green emergent species. Deer may be infected while feeding on emergent aquatic species in shallow water marshes and

wetlands throughout the year. This conclusion is supported by observations of white-tailed deer feeding almost exclusively from the shoreline and rarely entering water bodies beyond more than a few centimeters depth (Fig. 5; Hennings 1977).

White-tailed deer, moose, and elk forage on emergent *Equisetum* spp. and graminoids (Hunt 1979, Collins and Urness 1983, Jenkins and Starkey 1993). As in most cervids, foraging on emergent vegetation is likely related to seasonal dietary restrictions and nutritional stress. After the onset of lactation, female elk in Washington were more closely associated with riparian areas and small stream beds (McCorquodale et al. 1986). Elk, in general, are more closely associated with some aquatic habitats, such as wet meadows, than other sympatric cervids (Collins and Urness 1983), which may result in higher intensity infections in elk.

Geophagic activity also represents a potential infection source. Many cervids have been documented eating soils or using mineral licks (Weeks and Kirkpatrick 1976, Fraser et al. 1984, Abrahams 2013, Lavelle et al. 2014). Recent evidence suggests that sodium and phosphorus are likely the sought after nutrients at mineral licks for mountain goats, although other dissolved minerals are also present (Slabach et al. 2015).The dissolved minerals in these soils could create a rich habitat for snails and represent a shared resource between *F. magna* definitive and dead-end hosts, such as moose (Lavelle et al. 2014). Snail species in these habitats and shared use of these resources by white-tailed deer and other cervids warrants further investigation.

The historic and current status of *Fascioloides magna* in North American cervids

F. magna populations in elk and caribou appear to be expanding in Alberta and Labrador, Canada, respectively (Fig. 6). Increases in fluke prevalence in various white-tailed deer populations (Fig. 7) and Minnesota's northwestern and northeastern moose populations are also evident (Fig. 6). In free-ranging moose collected from northwestern and northeastern Minnesota between 2003 and 2013, 60% of animals were infected with *F. magna* (Wünschmann et al. 2015). In northeastern Minnesota moose, 58% of the examined individuals were infected (32 of 55). This is an increase of about 40% over ~40 years (17% in 1971; Karns 1972) and 50% over ~80 years (4% in the 1930s; Fenstermacher and Jellison 1933, Fenstermacher 1934, 1937). Although prevalence varies widely by location (Stromberg et al. 1983), the infection prevalence in white-tailed deer has only increased from ~20% to 30% over the past 70 years (Olsen and Fenstermacher 1943, Van der Waal et al. unpublished data, Vannatta and Moen *in prep*). These trends suggest that moose and white-tailed deer habitat overlap is increasing and that some level of parasite self-regulation or natural host immunity may be present in white-tailed deer (Foreyt et al. 1977). This is quickly becoming an important issue as climatic and anthropogenic alterations will likely lead to increased deer-moose overlap in the near future (Waller and Alverson 1997, Galatowitsch et al. 2009).

It appears likely that higher deer densities would contribute to infection risk in moose. However, there is little direct evidence to support this. Foreyt et al. (1977) concluded that *F. magna* may only reach a threshold prevalence in populations. The infection prevalence appears to plateau at ~70% in host deer populations, suggesting

~30% of the deer populations may have resistance to *F. magna* infection (Foreyt et al. 1977). For example, white-tailed deer in Texas consistently have infection prevalences near 70% (Fig. 7). Kootenay National Park's elk population plateaus near 75 or 80% infection, whereas moose plateau near 90% infection in Minnesota (Fig. 6). These higher values could represent host-parasite coevolution manifesting as higher resistance in natural host populations.

Deer likely have a longer evolutionary history with flukes than elk or moose have. This hypothesis is supported by recent genetic evidence, which suggests *F. magna* populations were divided into a western (Pacific northwest and Alberta) and eastern (southern USA, Great Lakes, Labrador) population and have subsequently diverged (Bazsalovicsová et al. 2015). The divergence patterns seen in regional *F. magna* populations is likely related to many factors, including glaciation, expansion of the Great Plains, extirpation of deer and subsequent restocking programs (Bazsalovicsová et al. 2015). The genetic isolation of some *F. magna* populations likely led to more specialization between regional parasites and hosts. As a consequence, we cannot assume that one regional population of *F. magna* when introduced to a new location will behave in a similar fashion to the current parasite population.

Within deer in northeastern Minnesota, infection prevalence is highest around Duluth, MN, USA and decreases toward the northeast suggesting deer and/or flukes are less established in these areas (Fig. 8A, B; VanderWaal et al. unpublished data). However, infection prevalence never falls to 0% in any area, meaning moose are at risk

of infection wherever deer are present. Consequently, an understanding of deer-fluke relationships is necessary for a comprehensive analysis of infection risk in moose.

A number of studies have demonstrated that *F. magna* infection prevalence increases with age class in cervids (Fig. 9). Thus, age structure of sympatric cervid populations could affect infection risk in moose. Additionally, the home range composition of sympatric deer appears to affect infection risk. Various authors show an increase in wet cover types leads to an increase in infection risk for ungulates (Mulvey et al. 1994, Vanderwaal et al. 2014, Pybus et al. 2015, Vannatta and Moen *Ch. 3*). Vanderwaal et al. (2014) linked this infection risk to rooted and floating aquatic marshes, which are known to contain many aquatic plant species palatable to both deer and moose (Hop et al. 2001).

Future directions

To make informed management decisions, researchers must understand the impact *F. magna* has on host species. The most important question for *F. magna* in moose is what degree of damage giant liver flukes actually cause. The answer to this question has been elusive. Some studies have suggested that white-tailed deer, a natural host which is thought to sustain little to no harm from infection, may suffer increased mortality when infected with *F. magna* (Cheatum 1951, 1952, Addison et al. 1988). Cheatum (1951) found a significantly higher fluke infection prevalence from winter-killed deer compared to deer collected by researchers. Winter-killed deer were twice as likely to have liver fluke infections compared to collected deer. The cause of this is not fully understood, but

immune response, anemia, eosinophilia, and hepatic tissue damage associated with fluke migration are likely energetically costly in stressful periods (Presidente et al. 1980).

Flukes may cause mortality indirectly through hepatic hemorrhage during predation events (Cowan 1946). In one report, nine deer died after being chased by domestic dogs (Cowan 1946). Necropsy after the chase suggested a combination of heavy fluke infection and exertion caused the hepatic portal system to hemorrhage. Fluke capsules are often near major blood vessels in the liver (Vannatta and Moen *Ch. 3*). Hepatic hemorrhage during chase events could explain some differences between the northwestern and northeastern Minnesota moose populations. Moose in northwestern Minnesota experienced little direct predation mortality (~3%; Murray et al. 2006). In contrast, predation accounts for ~34% of mortality in northeastern Minnesota (Minnesota DNR 2016). This could explain why the northwestern moose population continued to increase even with fluke prevalence near 90% despite infected moose appearing outwardly healthy.

Moderate to marked liver damage from fluke infection was not correlated with body condition in Minnesota moose (Wünschmann et al. 2015). However, 42% of malnourished moose had marked liver damage (>50% of the tissue altered by flukes) compared to 13% of animals in moderate and 33% of individuals in good condition. The sample size of individuals in good condition was small (N=8) compared to moderate and malnourished individuals (N=24 and 28, respectively), but these results hint at a possible pattern. The inconclusive nature of these studies suggests an energetic cost-of-infection analysis in moose is a pressing research need. Immune responses and tissue repair costs

are difficult to measure directly, but by measuring the resting metabolic rate of moose infected and uninfected with *F. magna* an energetic cost estimate may be possible (Robar et al. 2011). Another indirect measure of immune cost could be lactation. In cattle infected with the closely related fluke, *Fasciola hepatica*, Ross (1970) found a significant negative impact of fluke burden on milk yield. This represents a mechanism by which heavy fluke burdens in seemingly healthy adult moose could be related to fitness beyond direct mortality.

Fitness effects outside of direct mortality have been documented in white-tailed deer in South Carolina (Mulvey and Aho 1993, Mulvey et al. 1994). After controlling for habitat and year to year variation, male white-tailed deer with heavy fluke infections had fewer antler points and lower body weights (Mulvey and Aho 1993). In older bucks these effects were less dramatic or absent. However, old bucks with heavy fluke infections lost significantly more weight during the rut than uninfected individuals, which could decrease overwinter survival (Mulvey and Aho 1993). Fluke-infected female deer had higher body weights before the onset of breeding season and, thus, conceived earlier and outside of the optimal breeding window. Mulvey et al. (1994) suggested that the lower probability of twinning in infected does allowed for rapid weight gain, earlier breeding receptivity, and earlier conception. In both male and female deer, outward symptoms of infection would be minimal, but the fitness of infected individuals was likely altered. Similar studies in moose would be helpful in determining the true fitness cost of infection in this dead-end host.

On a broader scale, *F. magna* is prevalent in many regions of North America and represents a continent wide concern (Fig. 10, 11). A nation-wide risk assessment of *F. magna* infection risk would be useful to managers. In order to accomplish this, several basic biological questions must be answered. First, a comprehensive study describing the primary snail host for *F. magna* in each region is needed. Many experimental infections have been undertaken, but these often restrict miracidia to small areas and penetration of a less preferred host may be induced (Griffiths 1973, Foreyt and Todd 1978). Second, a fine scale analysis of habitat requirements for suitable snail hosts should be done. Some research has indicated that plant associations and hydrologic traits (e.g. pH, dissolved oxygen, water speed) may dictate the distribution of many snail hosts (Laursen et al. 1992, Laursen and Stromberg 1993). Additionally, GIS models show promise for predicting infection risk, but the presence of snail hosts in vernal pools poses a significant barrier to modelling snail habitat in areas with thick canopy cover (Malone and Zukowski 1992, DeRoeck et al. 2014). Lastly, aquatic feeding by cervids is not fully understood. An improved understanding of factors dictating the timing and degree of aquatic plant consumption and geophagy would aid managers in controlling exposure to *F. magna* metacercariae.

Before these critical knowledge gaps are addressed, management recommendations must be made with caution. Careful monitoring of deer sympatric with moose and distribution of deer harvest to maintain deer population densities below 5 deer/ km^2 would benefit moose (Lankester 2010). Additionally, care is needed when transporting domestic and game animals for sport or reintroduction. *F. magna* has

become an invasive species in Europe through the transport of game animals and is a major pest in livestock in Europe and North America (Stromberg et al. 1983, Králová-Hromadová et al. 2011). Transportation of *F. magna* explains much of its patchy geographic range in North America (Pybus 2001, Bazsalovicsová et al. 2015). The invasive potential of *F. magna* dictates strenuous monitoring of individual animals that may be used for reintroduction. Simply examining feces of individuals for fluke eggs at one time point will not be an effective control measure. Flukes require, at a minimum, three months to reach maturity and begin shedding eggs (Erhardova-Kotrla 1971). In white-tailed deer, fluke maturity and egg shedding did not begin until seven months after infection (Foreyt and Todd 1976a). In some instances, an infected individual may not shed eggs for years (Blazek et al. 1972). Animals prepared for reintroduction should be monitored for clinical signs of immature infection (Presidente et al. 1980), egg shedding, and should be given an anthelminthic to treat undetected infections (Foreyt and Todd 1973, 1976b, Qureshi et al. 1989, 1994, Pybus et al. 1991, Pybus 2001). This issue is of increasing importance, as elk reintroduction is being discussed in Minnesota, where moose are in decline (DelGiudice 2016).

The complex life cycle of *F. magna* creates issues when trying to manage disease. In wildlife populations, control measures such as vaccination, molluscicides, wetland draining, introducing competitor snails, or selective breeding are not cost effective nor 100% effective (Holland 1959). Qureshi et al. (1994) were able to lower prevalence of *F. magna* in white-tailed deer in Texas using medicated bait, but it was not eradicated after 3 years of treatment in a relatively small area. In areas of management need, *F. magna*

control must be examined from both the abiotic and biotic perspective. However, research on the efficacy of these management options should only be undertaken once the fitness cost of *F. magna* in the cervid of concern is known.

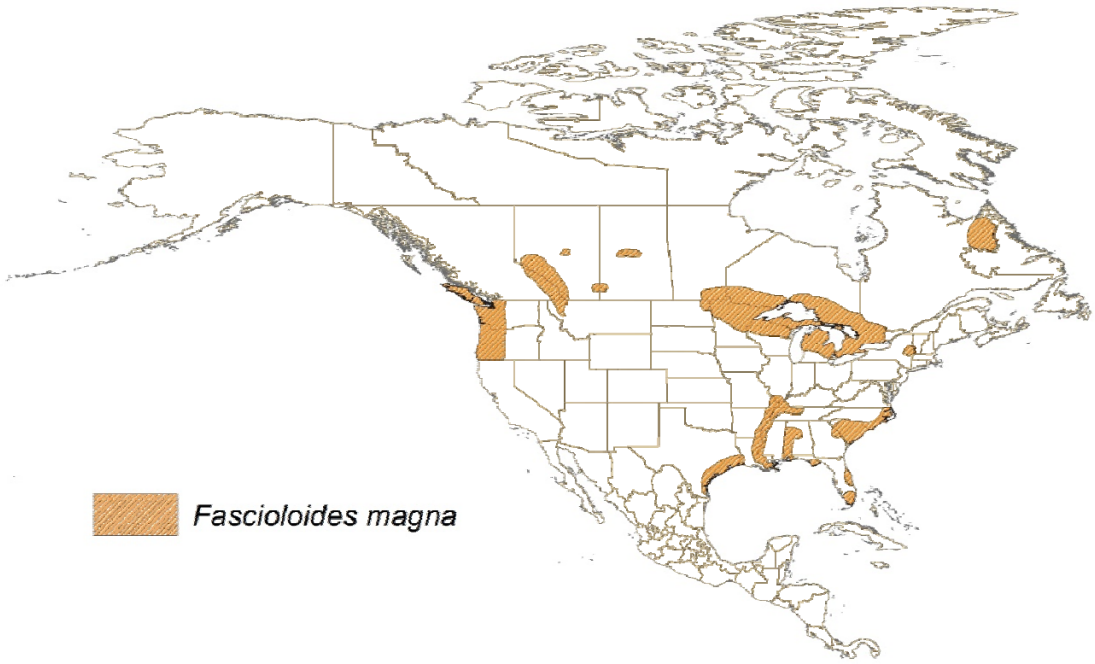

Figure 1. North American distribution of *Fascioloides magna* redrawn from Pybus (2001). North Dakota and Tennessee distributional information added from Maskey (2011) and Pursglove et al. (1977).

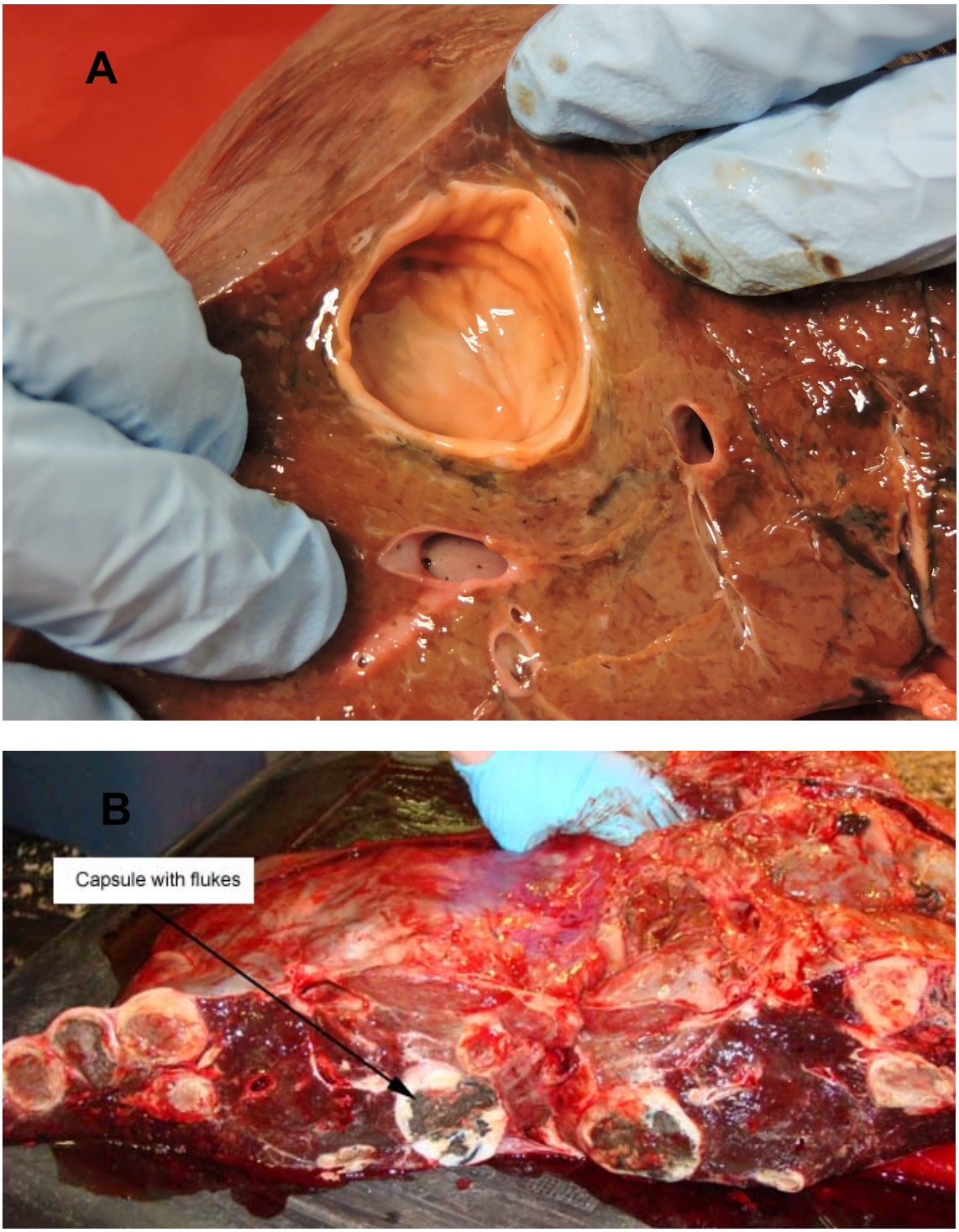

Figure 2. *Fascioloides magna* liver capsules in naturally infected (A) white-tailed deer and (B) moose. In deer, capsule walls are thin and material within the capsule is often fluid. In moose, capsule walls are thick and capsule material is solid or viscous (moose liver photo: Michelle Carstenson, Minnesota DNR).

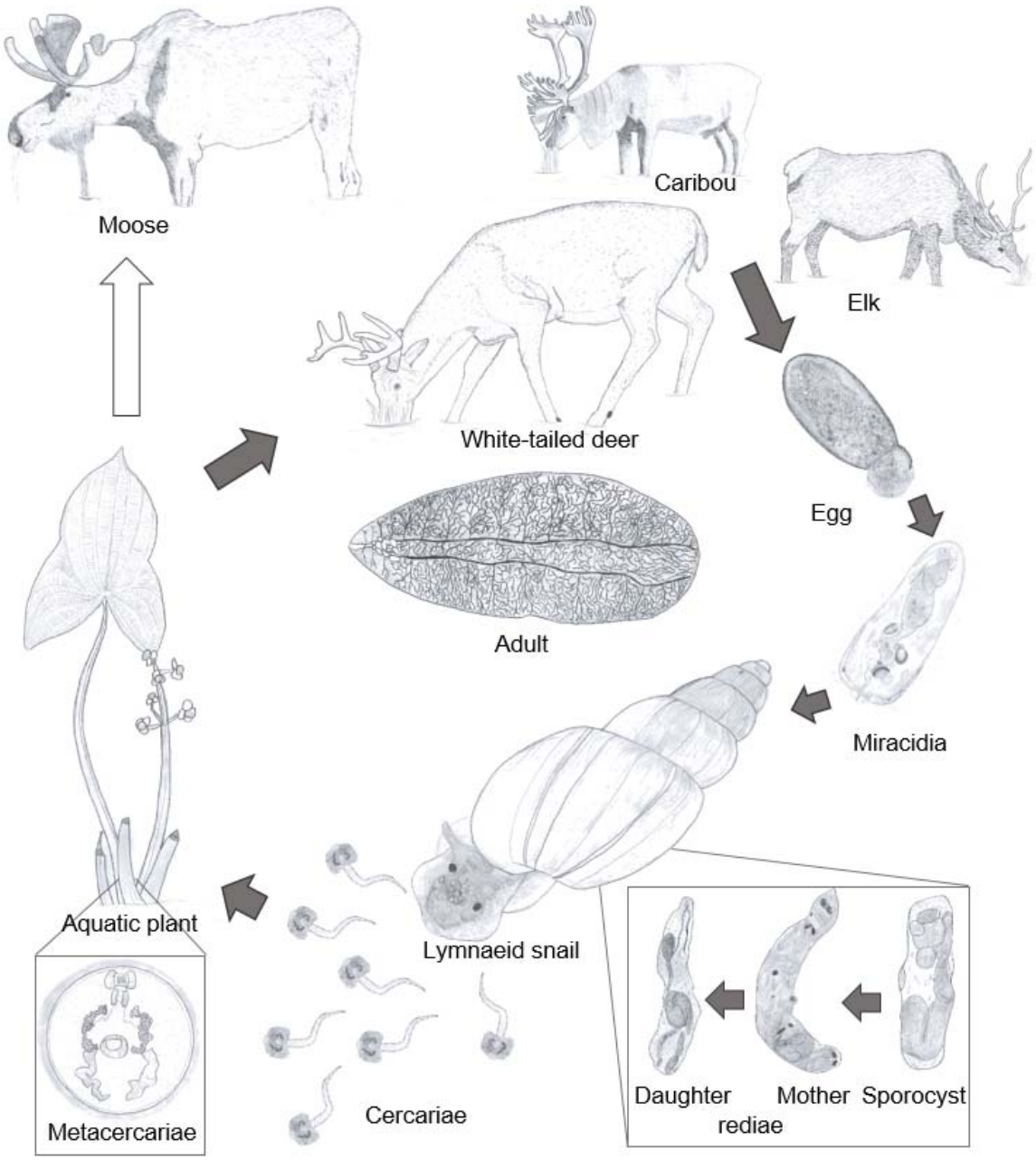

Figure 3. *Fascioloides magna* life cycle. Gray arrows indicate the normal life cycle progression. The hollow arrow indicates the dead-end host, moose, in which the parasite does not successfully shed eggs.

Table 1. Known intermediate snail hosts for *Fascioloides magna* in North America, common synonyms[a], and whether snails are known natural or experimental hosts.

***F. magna* host snail**	**Common synonyms**	**Natural or Experimental host (Source)**
Fossaria spp.	*Lymnaea humilis; L. bulimoides; L. modicella; L. parva; L. ferruginea; L. obrussa; L. umbilicata; L. desidiosa; L. dalli*	*Natural* (Swales 1935, Laursen and Stromberg 1993) *Experimental* (Sinitsin 1930, Krull 1933, Dutson et al. 1967, Griffiths 1973, Foreyt and Todd 1978)
Lymnaea caperata	*Stagnicola caperata; S. caperatus*	*Natural* (Griffiths 1962, Laursen and Stromberg 1993) *Experimental* (Griffiths 1962, Foreyt and Todd 1978, Laursen 1993)
Lymnaea elodes	*Stagnicola elodes; S. palustris; S. exilis; Lymnaea palustris; L. reflexa; L. umbrosa; S. reflexa*	*Natural* (Swales 1935) *Experimental* (Campbell and Todd 1955b, 1956, Griffiths 1962, Dutson et al. 1967, Foreyt and Todd 1978, Laursen 1993)
Lymnaea stagnalis	None	*Experimental* (Wu and Kingscote 1954, Griffiths 1973, Foreyt and Todd 1978)
Lymnaea catascopium	*Stagnicola catescopium; Lymnaea emarginata; L. woodruffi; L. walkeriana*	*Experimental* (Laursen 1993)
Lymnaea columella	*Pseudosuccinea columella*	*Experimental* (Krull 1933, Dutson et al. 1967[b], Laursen 1993, Flowers 1996)
Lymnaea cubensis	None	Host of closely related fluke, *Fasciola hepatica*, in Latin America (Cruz-reyes and Malek 1987)
Lymnaea megasoma	*Bulimnea megasoma*	12 specimens were found shedding gymnocephalus cercariae in Minnesota, presumably *F. magna* or *F. hepatica*. (Gilbertson et al. 1978)
Acella haldemani	*Lymnaea gracilis*	Not evaluated

[a] Synonyms derived from (Baker 1911b, Hubendick 1951, Clarke 1973, Dillon Jr. et al. 2013)
[b]This account is far outside the known range of *L. columella* and in doubt.

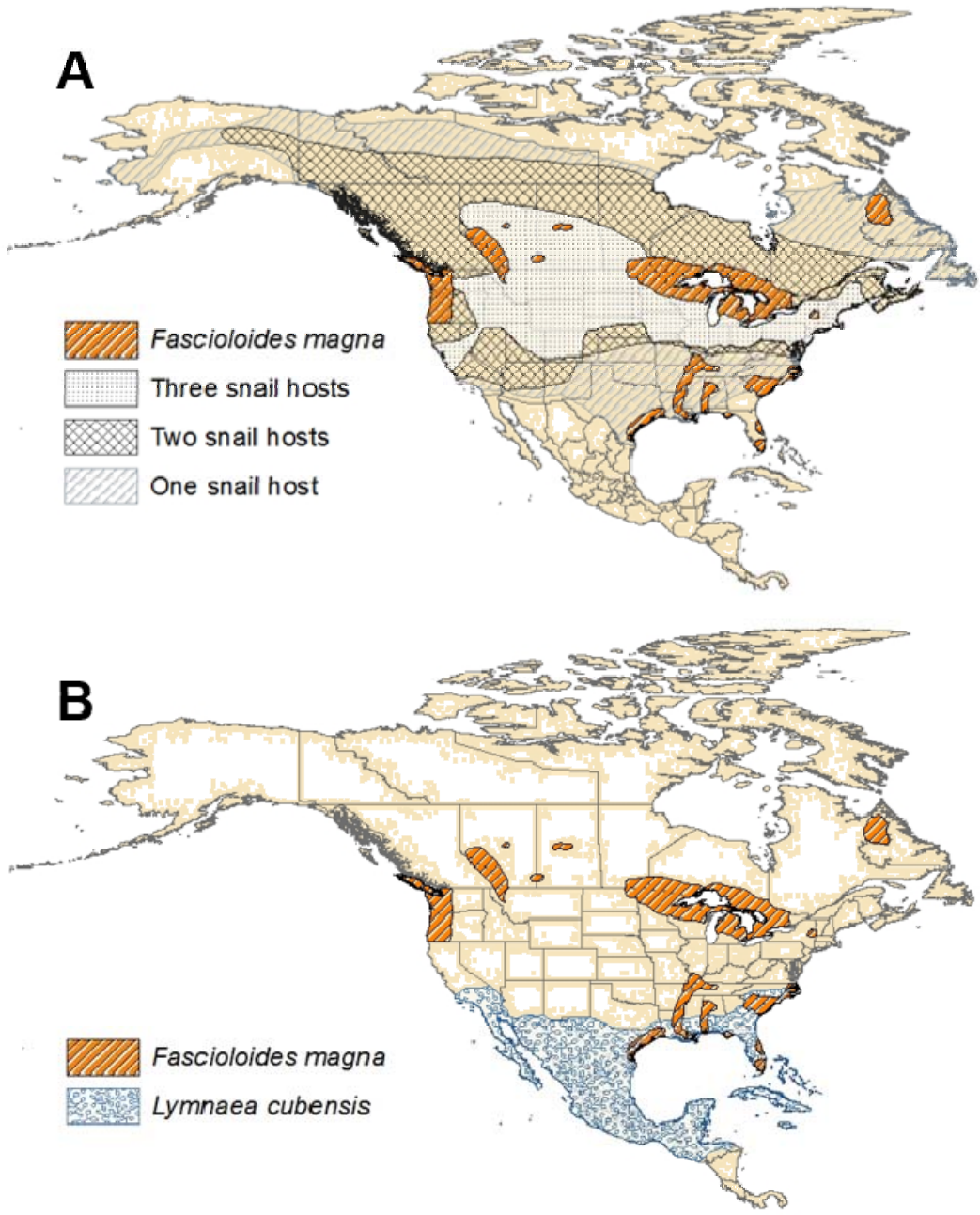

Figure 4A, B. (A) Geographic range of lymnaeid snail intermediate hosts in North America. Different patterns represent the number of known, natural intermediate host snails in an area. (B) Geographic range of *L. cubensis*, a potential host in Florida and the gulf coast. Maps derived from The IUCN Red List of Threatened Species, Baker 1911a, Hubendick 1951, Clarke 1973, 1981, Jokinen 1992, Laursen et al. 1992, and Pybus 2001.

Table 2. Known *Fascioloides magna* hosts and their habitat associations.

***F. magna* host snail**	**General habitat description**	**Source**
Fossaria spp.	Highly amphibious. Found on mud substrates in vernal pools and ditches.	(McCraw 1959, 1961, Clarke 1981, Laursen et al. 1992, Laursen and Stromberg 1993, Dunkel et al. 1996)
Lymnaea caperata	Found in floodplains, marshes, and other temporary waters. Can be tolerant of dry conditions.	(Clarke 1981, Laursen et al. 1992, Laursen and Stromberg 1993, Dunkel et al. 1996)
Lymnaea elodes	Found among vegetation in many water bodies. Primarily lakes, streams and ponds	(Clarke 1981, Laursen et al. 1992, Dunkel et al. 1996)
Lymnaea stagnalis	Found on vegetation (e.g. *Typha*) and rocks in large, permanent lakes with diverse substrates.	(Clarke 1981, Laursen et al. 1992, Dunkel et al. 1996)
Lymnaea catascopium	In lakes or large, slow moving rivers on rocks or vegetation exposed to current.	(Clarke 1981, Laursen et al. 1992, Dunkel et al. 1996)
Lymnaea columella	Found in lakes and other slow moving waters on vegetation or submerged sticks.	(Clarke 1981)
Lymnaea cubensis	Found in ponds and marshes likely similar to *Fossaria* spp.	(Hubendick 1951, Pointier and Augustin 1999)
Lymnaea megasoma	Occurs in many habitats from large to small lakes, beaver ponds, and slow moving rivers on muddy and silty sediments	(Clarke 1981, Vannatta and Moen *Ch. 2*)
Acella haldemani	An exceptionally rare species found in shallow water vegetation of ponds and lakes	(Clarke 1981)

Table 3. Representative aquatic vegetation consumed by moose.

Emergent	Floating/Submergent	Algae
Carex spp.	*Brasenia schreberi*	*Spirogyra*
Equisetum spp.	*Myriophyllum verticillatum*	
Glyceria borealis	*Nuphar variegatum*	
Menyanthes trifolium	*Nuphar advena*	
Sagittaria spp.	*Nymphaea odorata*	
Sparganium angustifolium	*Potamogeton alpinus*	
Zizania spp.	*Potamogeton ampifolius*	
	Potamogeton epihydrus	
	Potamogeton filiformis	
	Potamogeton foliosus	
	Potamogeton gramineus	
	Potamogeton pusillus	
	Potamogeton natans	
	Potamogeton perfoliatus	
	Potamogeton praelongus	
	Potamogeton richarsonii	
	Potamogeton zosteriformis	
	Scirpus subterminalis	
	Utricularia vulgaris	
	Vallisneria americana	
	Nitella and *Chara*	

Data from: (DeVos 1958, Hennings 1977, Belovsky and Jordan 1981, Fraser et al. 1984)

Table 4. Representative aquatic vegetation consumed by white-tailed deer.

Emergent	Floating/Submergent	Algae
Carex spp.	*Brasenia schreberi*	*Spirogyra*
Dulichium grundinaceum	*Ceratophyllum demersum*	
Eleochris spp.	*Myriophyllum* spp.	
Equisetum fluviatile	*Nuphar variegatum*	
Hypericum boreale	*Nymphaea odarata*	
Potentilla palustris	*Potamogeton epihydrus*	
Sagittaria spp.	*Potamogeton* spp.	
Sium suave	*Polygonum* spp.	
Sparganium spp.	*Polygonum amphibium*	
Typha spp.	*Utricularia* spp.	
Zizania aquatica		
Gramineae		
Cyperaceae		
Juncaceae		

Data from: (Dahlberg and Guettinger 1956, Harlow and Jones Jr. 1965, Skinner and Telfer 1974, Hennings 1977, Watkins and Ullrey 1983)

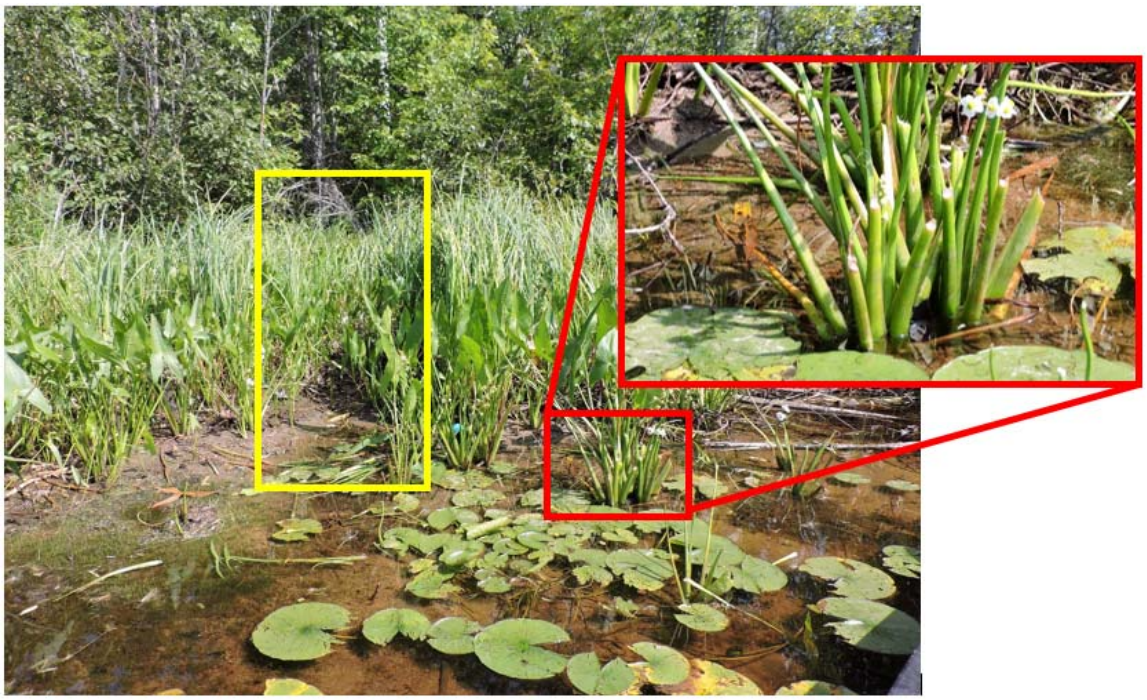

Figure 5. Example of white-tailed deer aquatic forage. The left box outlines the deer path leading to the water's edge. The right enlarged image shows deer browse on *Sagittaria* spp. leaves.

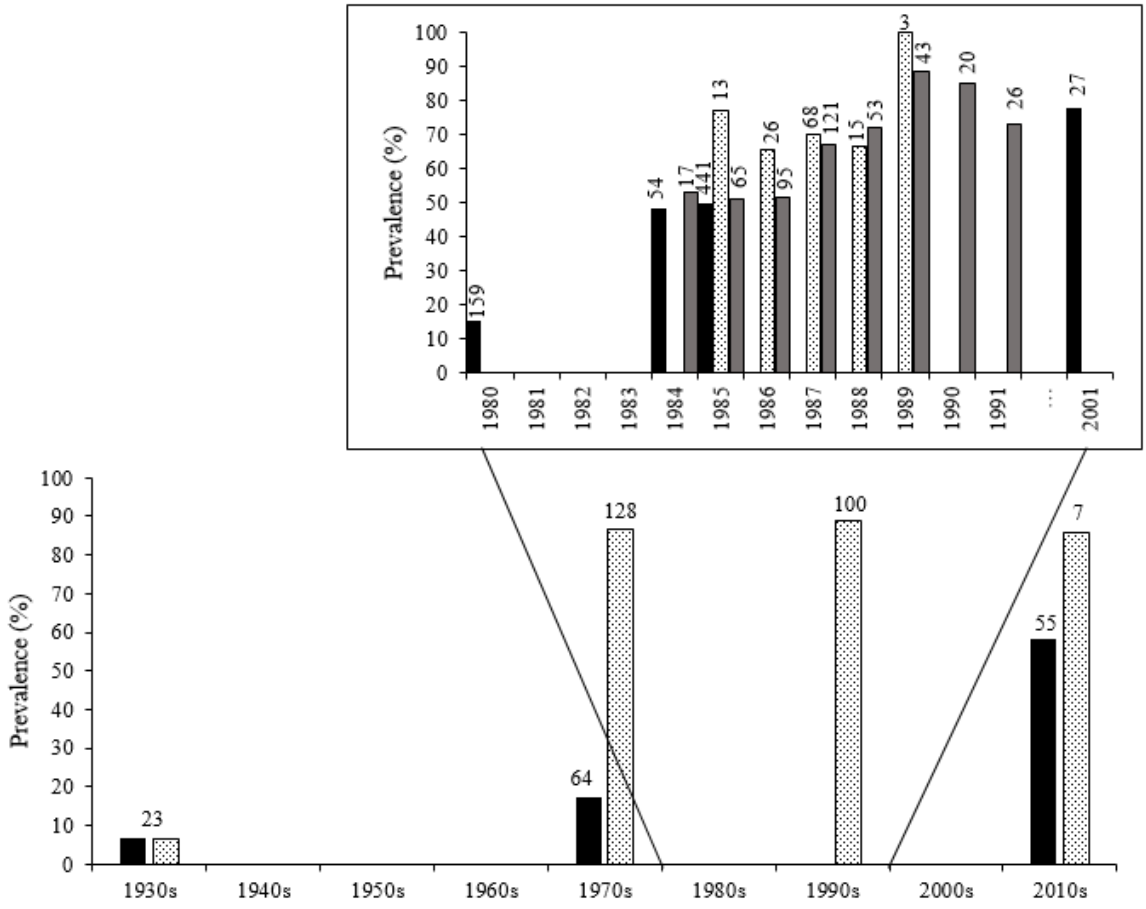

Figure 6. Historic prevalence of *F. magna* infections in moose in northeastern Minnesota (black) and northwestern Minnesota (speckled). Inset shows caribou in Labrador, Canada (black), elk in Kootenay National Park, Alberta, Canada (speckled), and elk in Banff National Park, Alberta, Canada (gray). Numbers above each bar represent study sample size. Data from: (Fenstermacher and Jellison 1933, Fenstermacher 1934, Wallace 1934, Fenstermacher 1937, Karns 1972, Parker 1981, Huot and Beaulieu 1985, Lankester and Luttich 1988, Pybus 1990, Pybus et al. 1991, 2015, Butterworth and Pybus 1992, Murray et al. 2006, Pollock et al. 2009, Wünschmann et al. 2015).

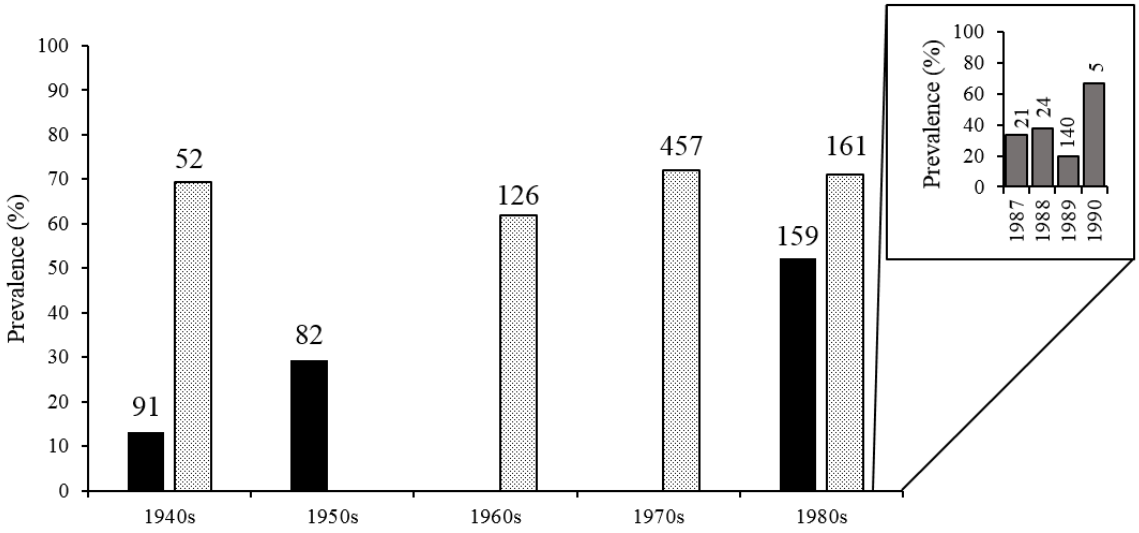

Figure 7. Historic prevalence of *F. magna* infections in white-tailed deer in Ontario, Canada (black), and Texas, USA (speckled). Inset shows the prevalence of *F. magna* in Alberta, Canada in the late 1980s. Numbers above each bar represent study sample size. Data from: (Olsen 1949, Kingscote 1950, Draper 1952, Glazener and Knowlton 1967, Foreyt and Todd 1972, Pursglove et al. 1977, Foreyt et al. 1977, Foreyt and Drawe 1978, 1985, Addison et al. 1988, Lydeard et al. 1989, Pybus 1990, Mulvey and Aho 1993, Qureshi et al.1994, Pybus et al. 2015)

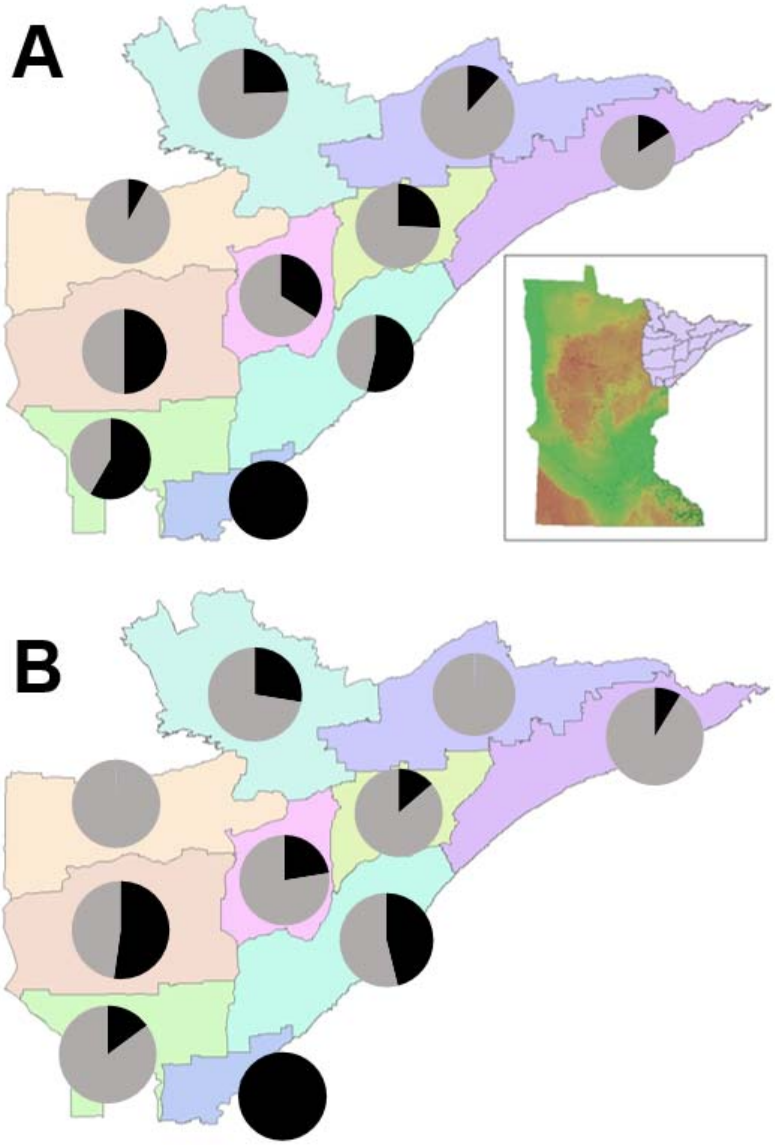

Figure 8. Deer permit areas (DPA) in Minnesota's arrowhead region (Inset: Minnesota digital elevation model with arrowhead region DPAs highlighted). (A) Infection prevalence decreases from the southwest to the northeast (black = proportion infected). (B) Mean infection intensity is less consistent across the region (black = mean infection intensity in each DPA as a proportion of the greatest mean, 787 eggs per gram dry weight feces; VanderWaal et al. unpublished data).

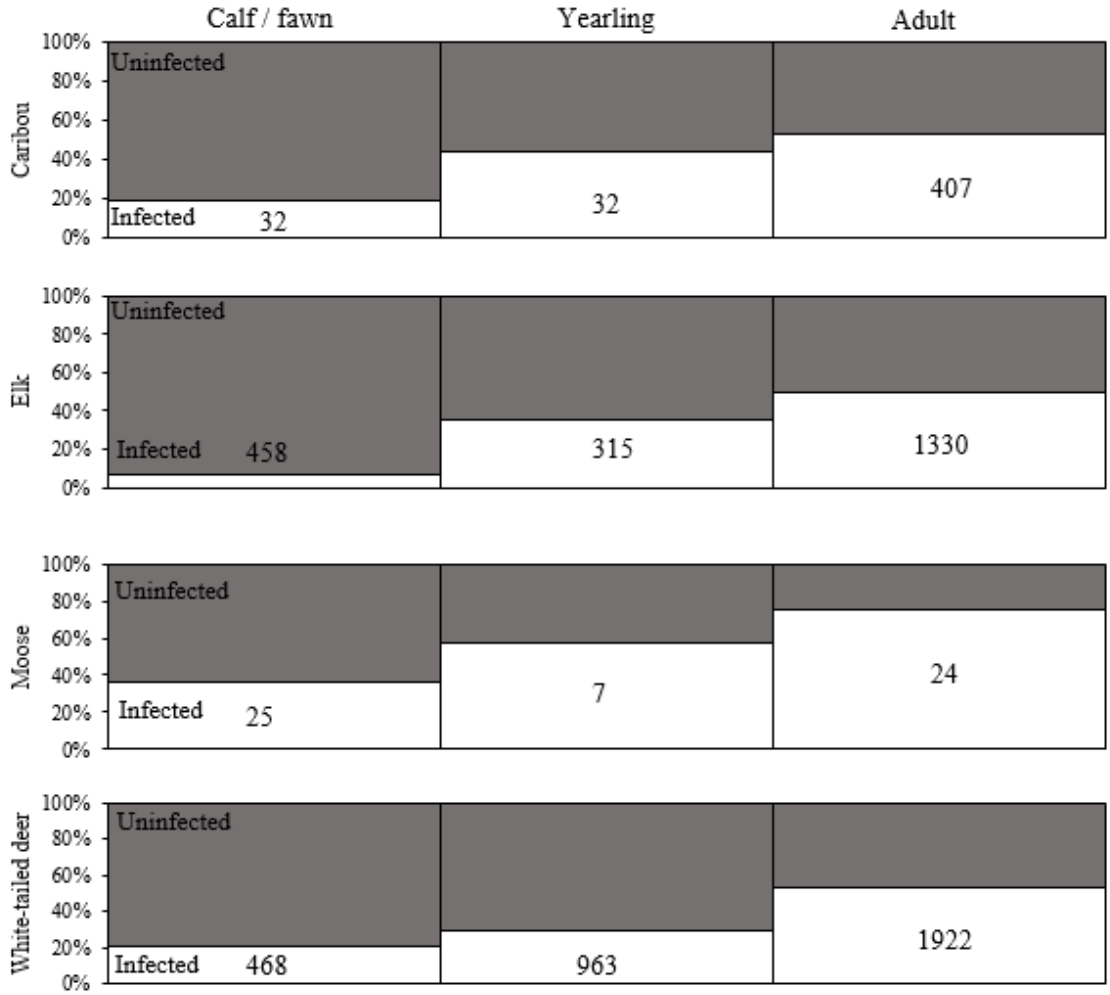

Figure 9. Percent of *F. magna* infected (white) and uninfected (gray) individuals in different age classes for different cervid species. Inset numbers represent sample size. Data from: (Cheatum 1951, Flook and Stenton 1969, Foreyt et al. 1977, Huot and Beaulieu 1985, Addison et al. 1988, Lankester and Luttich 1988, Pybus 1990, Butterworth and Pybus 1992, Laursen 1993, Mulvey and Aho 1993, Qureshi et al. 1994, Pybus et al. 2015, Wünschmann et al. 2015)

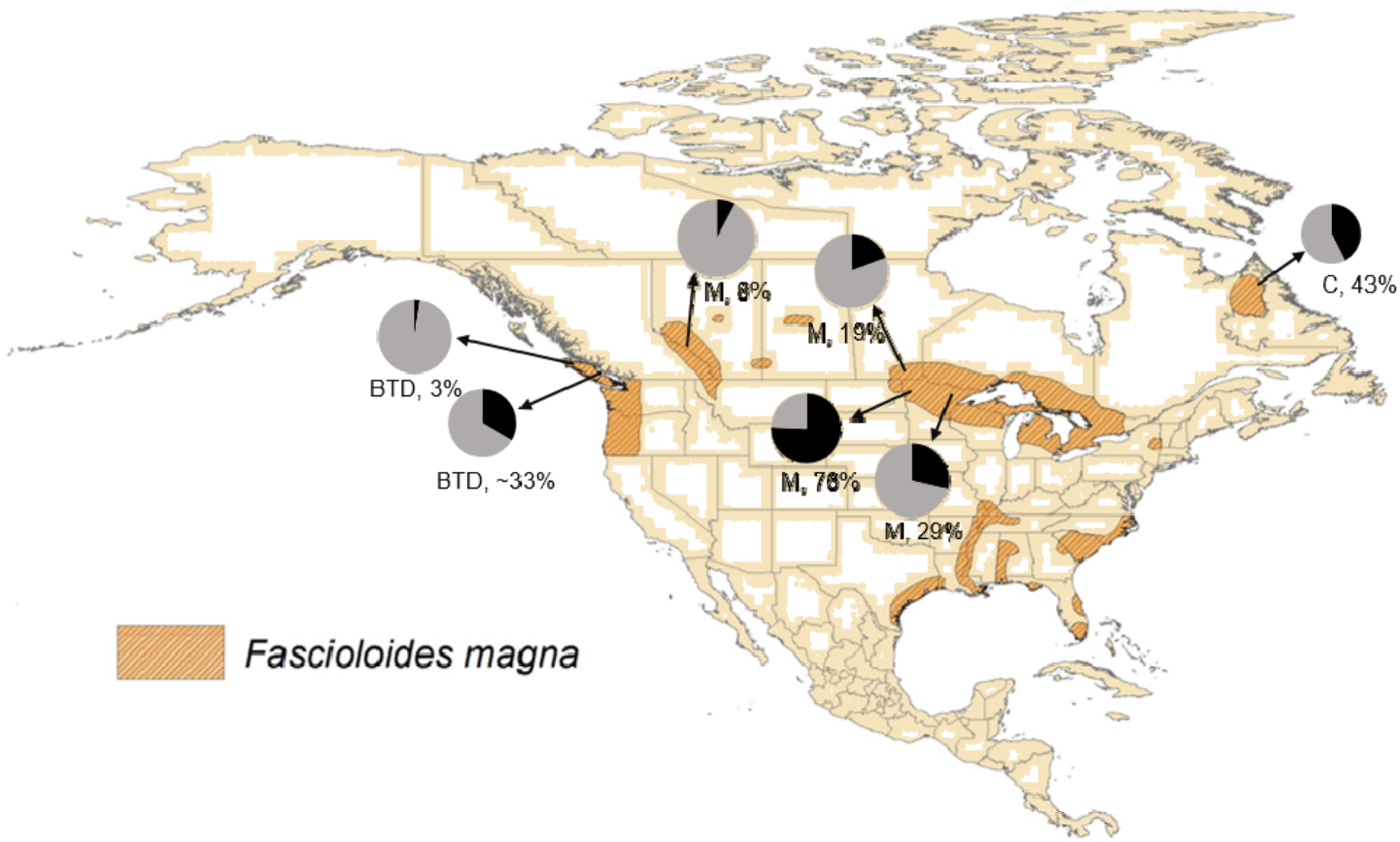

Figure 10. Average prevalence of *F. magna* in various cervid populations across North American (black = proportion infected; gray = proportion uninfected). Abbreviations represent moose (M), caribou (C), and black-tailed deer (BTD) followed by percent infected. Data from: (Kermode 1916, Fenstermacher and Jellison 1933, Fenstermacher 1934, Wallace 1934, Fenstermacher 1937, Cowan 1946, Karns 1972, Lankester 1974, Samuel et al. 1976, Parker 1981, Huot and Beaulieu 1985, Lankester and Luttich 1988, Pybus 1990, Murray et al. 2006, Pollock et al. 2009, Maskey 2011, Pybus et al. 2015, Wünschmann et al. 2015).

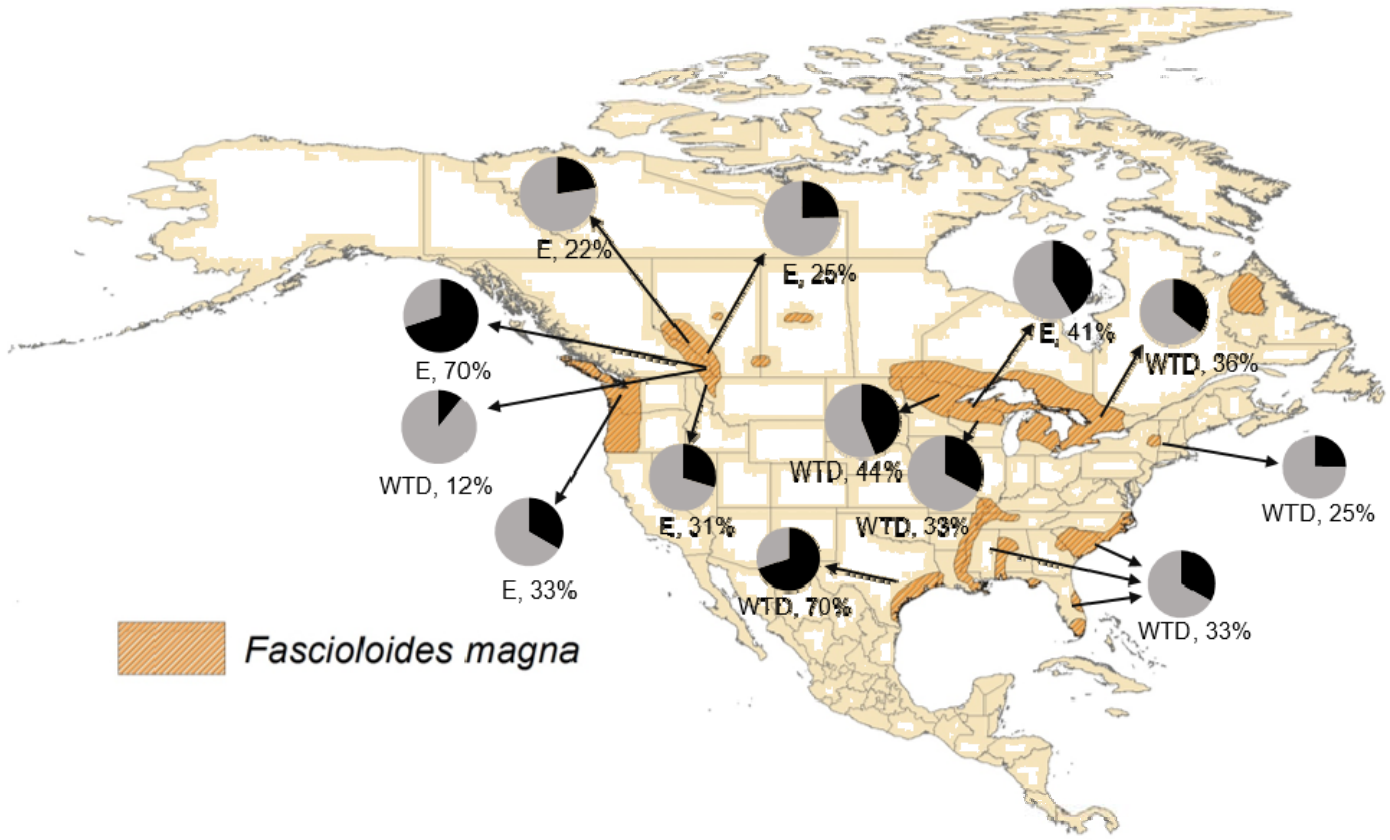

Figure 11. Average prevalence of *F. magna* in various cervid populations across North American (Black = proportion infected; gray = proportion uninfected). Abbreviations represent white-tailed deer (WTD) and elk (E) followed by percent infected. Data from: (Aiton 1938, Schwartz 1938, Dinaburg 1939, Olsen and Fenstermacher 1943, Fenstermacher et al. 1943, Olsen 1949, Kingscote 1950, Cheatum 1951, Draper 1952, Dahlberg and Guettinger 1956, Glazener and Knowlton 1967, Flook and Stenton 1969, Samuel and Trainer 1969, Foreyt and Todd 1972, 1976b, Foreyt et al. 1977, Pursglove et al. 1977, Davidson et al. 1981, Christian 1984, Addison et al. 1988, Lydeard et al. 1989, Pybus 1990, Pybus et al. 1991, 2015, Butterworth and Pybus 1992, Laursen 1993, Mulvey and Aho 1993, Qureshi et al. 1994, Flowers 1996, Weiland 2008, Vanderwaal et al. 2014)

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Chapter 2:

Lymnaeid Snail Distribution and Parasitism in Northeastern Minnesota with Special Reference to *Lymnaea megasoma* and *L. stagnalis*

Summary

Snails (gastropods) are an understudied and imperiled group of organisms. Basic biological information for these organisms is scant, despite their roles in nutrient turnover and as parasite intermediate hosts. We used a combination of field sampling, ordination techniques, grouping statistics, indicator species analysis, and univariate logistic regression to better describe the habitat of two common species, *Lymnaea megasoma* and *L. stagnalis,* in northeastern Minnesota, USA. Survival of snails in captivity was measured and correlated with presence of trematode parasites. The current study indicates *L. megasoma* was found in various stagnant water habitats from small bays in lakes to road side ditches and beaver ponds. *L. stagnalis* presence in an area is related to the proportion of open water habitat and suitable vegetation communities. *L. megasoma* was often infected with trematode cercariae and warrants further study as a parasite intermediate host in this area.

Introduction

Snails (gastropods) are among the most imperiled organisms on the planet (Lydeard et al. 2004, Johnson et al. 2013). However, more information is needed about the basic biology of these organisms (Dillon Jr. 2000). From a management perspective, these species represent important parasite intermediate hosts for many vertebrate and invertebrate taxa (Olsen 1974). On the ecosystem level, snails are important biomass contributors, nutrient recyclers, and prey for other species (Dillon Jr. 2000, Bernot 2013).

Among snail taxa, the lymnaeid family of snails is of particular importance. The Lymnaeidae host several trematode parasites of veterinary significance (Olsen 1974, Pybus 2001), are widely dispersed (Baker 1911a, Hubendick 1951, Clarke 1973, Dillon Jr. et al. 2013), and, paradoxically, 61% of the species in the family are imperiled (Johnson et al. 2013). Despite this group's importance, there is great uncertainty regarding the current phylogeny of lymnaeids (Correa et al. 2010, 2011).

Few studies have examined the distribution of freshwater gastropods in Minnesota (Baker 1911a, 1929, 1935, 1936, Dawley 1947, Clarke 1973, Laursen et al. 1992). Of these studies, none have examined fine scale snail distributions within the northeastern counties of St. Louis, Lake, and Cook.

In the current study, we examine the distribution of lymnaeid snails within St. Louis County and Lake County in northeastern Minnesota. Field and laboratory techniques were used to identify suitable lymnaeid snail habitat and the parasite communities within these snails. Particular emphasis was placed on comparing the habitats used by *Lymnaea stagnalis* and *L. megasoma*, a species underrepresented in the literature (Gilbertson et al. 1978, Jokinen 1992, McKillop et al. 1993).

Methods

Field sampling

Aquatic habitats were sampled across St. Louis County and Lake County in Minnesota in 2015. Locations were selected for accessibility such as public water access or proximity to roads and trails. 84 sampling sites were surveyed by hand and dip net for lymnaeid

snails (Fig. 1). Water characteristics such as pH, water hardness as $CaCO_3$, and water surface temperature were measured. Additionally, a 1 m^2 vegetation quadrat was taken at a random point in each location and around each snail. Sampling points were buffered by 10, 25, 50, and 100 m and analyzed for cover type composition using ArcMap (v10.3, ESRI Inc., Redlands, CA) and the Geospatial Modelling Environment (v0.7.4.0, Dr. Hawthorne L. Beyer, Spatial Ecology LLC). Land cover data came from the National Land Cover Database 2011 (NLCD; U.S. Geological survey) and Land Use Land Cover (LULC; USGS) raster datasets, both at 30 m resolution. Lake characteristics such as fetch, width:length ratio, and littoral zone area were obtained from the DNR LakeFinder (URL: http://www.dnr.state.mn.us/lakefind/index.html), the Minnesota Geospatial Commons (URL: https://gisdata.mn.gov/), or measured using aerial imagery in ArcMap (e.g. ponds, vernal pools, and beaver ponds).

Ordination analyses

Nonmetric multidimensional scaling (NMS) was done using percent cover vegetation quadrats for each snail in PC ORD 6 (Kruskal 1964a, 1964b, McCune et al. 2002). Snail species and cercariae groups were input as categorical environmental variables in the site X environment matrix. A random distribution of points was used as the starting configuration for NMS and 50 runs were completed on the real data. We used relative Sorenson distances for all ordination analyses. Only lakes, vernal pools, and beaver ponds were included in ordination analyses in order to conserve comparable environmental variables such as lake area, littoral zone area, fetch, etc. Variables which

were not available for all sampling locations were excluded from ordination analyses. These include volume development index, island shoreline length, chlorophyll a, total phosphorus, water clarity, and others.

Multi-response permutation procedures (MRPP) were used to test for differences between snail species groups. This technique compares weighted mean within-group distance measures, in this case relative Sorenson distance, determining if the within-group distance is less than expected by chance alone (Biondini et al. 1985, McCune et al. 2002).

Indicator species analysis (ISA) was used to compare the vegetation communities associated with *Lymnaea megasoma* or *L. stagnalis*. A contingency table test was used to determine if vegetation species and snail species group associations were greater than expected by chance (Tichy and Chytry 2006). The analysis was done using 4999 randomizations for a Monte Carlo test in PC ORD 6 (McCune et al. 2002).

Logistic regression analysis

Lymnaea megasoma and *L. stagnalis* presence/absence data was analyzed using univariate generalized linear models (GLM). Environmental variables were selected by comparing the means of sites with and without each snail species using a two sample Z test. Variables with p values < 0.1 were selected as candidates for logistic regression. Regressions were performed in R (v3.2.2, 2015 The R Foundation for Statistical Computing) using the glm() base function. Model goodness of fit was compared using

ΔAIC, Akaike weight, and the Hosmer and Lemeshow Goodness of Fit test for binary data (Wagenmakers and Farrell 2004).

Floristic Quality Index

The floristic quality index (FQI) associated with each snail quadrat and random quadrat was used in both the NMS analysis as an environmental variable and as a standalone regression. FQI was calculated two ways (Milburn et al. 2007, USGS 2011):

$$FQI_1 = \bar{C}\sqrt{S}$$

and

$$FQI_2 = \left(\frac{\sum(\%_i \times C_i)}{100}\right) \times 10$$

where C_i is the coefficient of conservatism for species i, $\%_i$ is the percent coverage of species i and S is the number of species within a quadrat.

Parasitism

Snails were collected and taken to the Natural Resources Research Institute in Duluth, Minnesota, USA. Snails were weighed and stored in 100 mL specimen containers until death. Containers were filled with lake water collected from sampling locations and stored in the dark at ~20 ºC. Water was replaced twice a week and snails were fed lettuce once a week. Twice weekly, snails were placed under artificial light to induce shedding of trematode cercariae (Caron et al. 2008). Cercariae were stained with neutral red

(0.001%) and identified (Schell 1970). After death, length and width measurements were taken for all snails (Perez et al. 2004). Snails which died within one week of capture were considered capture related mortalities and were not included in survival comparisons.

Results

Field sampling and landscape analysis

Lymnaea megasoma and *L. stagnalis* were the most widely distributed species (Fig. 2; N = 24 sites and N = 11 sites, respectively). *L. elodes*, *Fossaria* spp., and *L. catascopium* were also found, but in few locations (4, 3, and 1, respectively). *L. megasoma* was found in several habitats including lakes (63% of occurrences), beaver ponds (17%), streams and rivers (12%), and road side ditches (8%). This species was associated with organic substrates and stagnant water. *L. stagnalis* was found in lakes (91%) and one river, and in association with several substrate types including organic, rock, mud, and sand. *L. elodes* was found in three lakes and one roadside ditch. *Fossaria* spp. were found at two locations in a stagnant stream and one roadside ditch on mud or silt.

L. megasoma occurred in areas with less open water and more developed open space than areas without this species (Fig. 3). *L. stagnalis* occurred in areas with more open water than areas without this species (Fig. 4) and in areas with more open water than *L. megasoma* at a 100 m buffer using NLCD 2011 data (*L. stagnalis* mean = 0.47, *L. megasoma* mean = 0.27, two sample Z test, $Z_{32} = 2.10$, $p = 0.02$).

NMS, MRPP, and ISA analyses

After 108 iterations, the nonmetric multidimensional scaling configuration is displayed in three dimensional space with a final stress of 8.5 (Fig. 5; Kruskal 1964b, McCune et al. 2002). The stability criterion was a standard deviation in stress over the last 15 runs of 0.00001. Axis one had an $r^2 = 0.433$ for correlation between the ordination distances and original distances. Axis two and three had an $r^2 = 0.256$ and 0.174, respectively. A Monte Carlo test with 249 iterations gave a *p* value of 0.02.

Nonmetric multidimensional scaling (NMS) resulted in a clustering of points near the centroid of the NMS plot (Fig. 5). However, multi-response permutation procedure (MRPP) pairwise comparisons between snail species groups allows us to reject the hypothesis that there is no difference between groups (Table 1). ISA indicated that *Sagittaria* spp. and emergent grasses, sedges, and rushes were strong indicators of an *L. megasoma* site compared to an *L. stagnalis* site (Fig. 6). *Zizania* spp., *Potamogeton spirillus*, *Najas flexilis*, and *Potentilla* spp. were strong indictors of an *L. stagnalis* site.

Logistic regression analysis

For *L. megasoma*, six environmental variables were potential candidates for modelling presence/absence (Table 2). The proportion of open water within a 100 m buffer using NLCD land cover was the best model. However, the regression model based on $CaCO_3$ concentration also had substantial support ($\Delta AIC < 2$; Wagenmakers and Farrell 2004). Developed open space within a 100 m buffer was also a strong candidate model, but this was likely a result of sampling procedure, not snail ecology.

For *L. stagnalis*, five environmental variables were potential candidates for modelling presence/absence (Table 2). Lake width:length ratio was the best fit univariate model. The proportion of mixed forest within a 25 m buffer using NLCD land cover also had substantial support ($\Delta AIC < 2$), but this model was not considered since mixed forest contributed < 15% of the 25 m buffer and likely had little biological significance.

The FQI_1 and FQI_2 from each snail vegetation quadrat were regressed against quadrats taken at random locations within each sample site. In all cases, *L. megasoma* presence and *L. stagnalis* presence were negatively correlated with FQI (Table 3). However, both Hosmer and Lemeshow goodness of fit tests showed modelled FQI data was significantly different than observed data for *L. stagnalis*.

Parasitism

Trematode cercariae were found in few locations in St. Louis County and Lake County (Fig. 7A, B). Strigea cercariae were found at four sites, xiphidiocercariae found at three sites, and brevifurcate-apharyngeate cercariae were found at one site. *L. megasoma* had 6% and 10% infection rates with strigea and xiphidiocercariae, respectively. One *L. elodes* was found infected with strigea cercariae (25%) and one *L. stagnalis* was infected with brevifurcate-apharyngeate cercariae (1%).

The number of xiphidiocercariae shed by *L. megasoma* was not related to snail mass ($r^2 = 0.07$), length ($r^2 = 0.004$), or width ($r^2 = 0.04$). However, there was a weak positive correlation between *L. megasoma* width:length ratio and the number of xiphidiocercariae shed ($n = 8$, $r^2 = 0.51$, $y = 9911x - 5818$). A total of 3,217

xiphidiocercariae and 13,473 strigea cercariae were collected from *L. megasoma.* No snail morphometric measurement was correlated with strigea cercarial shedding.

Survival duration of *L. megasoma* infected with either xiphidiocercariae or strigea cercariae was lower than survival duration of uninfected snails (Fig. 8; one-tailed t test, $t_{47} = 3.50$, $p < 0.01$ for xiphidiocercariae infections and $t_{46} = 2.42$, $p < 0.01$ for strigea infections). Snail survival time was not different across infection types (t test, $t_9 = 0.57$, $p = 0.58$).

Discussion

Basic biological information for many snail species is sparse in the scientific literature (Dillon Jr. 2000). The current study is comparable to similar snail distribution and habitat studies near northeastern Minnesota or with a similar target group (Table 4). However, concurrent gastropod and parasite surveys have not been done in this area.

Distribution and landscape level habitat

L. megasoma was the most common and widely distributed lymnaeid snail (Fig. 2), occurring in lakes, rivers, streams, beaver ponds, and roadside ditches. *L. stagnalis* was also widely distributed, but more common in lake habitats. *L. megasoma* resides commonly in quiet back bays and beaver ponds that contain less open water within the 100 m buffer. *L. stagnalis* was found in large water bodies with high width:length ratios and higher proportions of open water compared to *L. megasoma* (Fig. 9).

Very little data is available on *L. megasoma*'s distribution in northern Minnesota (Dawley 1947, Clarke 1973, Gilbertson et al. 1978, Laursen et al. 1992, McKillop et al.

1993), but *L. megasoma* is restricted to the Great Lakes basin in North America (Baker 1911a, Hubendick 1951). Paradoxically, *L. megasoma* appears to have a wide environmental tolerance yet a restricted distribution (current study, Clarke 1981, Jokinen 1992). This range restriction is not likely due to dispersal barriers associated with snail size. Despite *L. megasoma* being the largest lymnaeid snail, *L. stagnalis* is comparable in size and is fairly ubiquitous across North America (IUCN Red List of Threatened Species). There may be some level of competition between *L. megasoma* and other lymnaeids or some unknown abiotic habitat restriction limiting species dispersal. This question warrants closer examination.

NMS, MRPP, and ISA

Support was found for distinct snail species groupings within the NMS ordination space using MRPP (Fig. 10). The *Fossaria* spp. group is located on the opposite end of the FQI joint plot line. *Fossaria* spp. are common in vernal pools, roadside ditches, and ephemeral wetlands which are prone to seasonal and human disturbances (McCraw 1959, 1961, Clarke 1981, Laursen et al. 1992). These areas would likely have lower FQI values and may suggest *Fossaria* colonize sites following disturbances. *L. stagnalis* and *L. megasoma* were both clustered near the centroid due to bare sediment being common within snail vegetation quadrats. However, *L. megasoma* showed a wider variety of habitat associations radiating outward with increasing proportions of shrub and grassland habitat (Fig. 10, 11). *L. stagnalis* had one cluster near the centroid at intermediate values of FQI and higher proportions of open water habitat. More diffuse points were dispersed

near the edge of the ordination space. These clusters may represent distinct *L. stagnalis* ecotypes. Other ecotypes of *L. stagnalis* have been recognized before (Baker 1911a, Hubendick 1951, Clarke 1973, 1981). A diversity of ecotypes may explain the wide distribution of *L. stagnalis* compared to *L. megasoma*. Ordination techniques should be explored further as a potential method to support inference on different snail species and ecotypes.

There are several possible indicator species for distinguishing between *L. stagnalis* and *L. megasoma* habitat. *Sagittaria* spp., *Nuphar* spp., *Elodea* spp., *Equisetum* spp., *Utricularia* spp., and emergent, terrestrial grasses, sedges, and rushes were the significant indicators of *L. megasoma* habitat compared to *L. stagnalis* habitat (Fig. 6). Taken together, these indicators suggest *L. megasoma* inhabits very shallow, slow-moving water with mucky soils in lakes, slow streams, beaver ponds, or bogs (Chadde 2012, Skawinski 2014). In contrast, *L. stagnalis* habitat was indicated by common littoral zone species, such as *Scirpus* spp. and *Najas flexilis* (Chadde 2012, Skawinski 2014). However, *Zizania* spp., which favors water 1-2 m in depth with a current, were also an indicator of *L. stagnalis* (Chadde 2012). *Zizania* is also found on diverse substrate types, as *L. stagnalis* was. It is unlikely that these indicator species represent higher quality forage for *L. megasoma* and *L. stagnalis* as these species are generalist herbivores and detritivores (Dillon Jr. 2000). Instead, these indicator species likely represent abiotic environmental conditions that are important for each snail species.

Logistic regression analysis

Univariate generalized linear models for *L. megasoma* suggested that both proportion of open water within a 100 m buffer and $CaCO_3$ concentration are likely significant predictor variables for *L. megasoma* presence/absence (Fig. 12A, B). However, *L. megasoma* abundance was not correlated with $CaCO_3$ concentration ($r^2 = 0.01$). This suggests calcium may act as a barrier to colonization, but not as a determinant of *L. megasoma* abundance (Dillon Jr. 2000).

The univariate FQI logistic regressions for *L. megasoma* and *L. stagnalis* showed species presence/absence was predicted by lower FQI values (Fig. 13A, B). The relationship between FQI and snail presence may suggest that *L. megasoma* and *L. stagnalis* colonize disturbed sites such as boat landings or private beaches. The validity of using FQI to measure this relationship is tenuous (Bried et al. 2012, 2013, Matthews et al. 2015). However, understanding the impacts of human disturbances on snail distribution warrants further study for this imperiled group.

L. stagnalis presence/absence was best modelled by lake width:length ratio with a positive coefficient (Table 2). Although not always true, lakes with a higher width:length ratio are likely to be round with a smaller shoreline development index (SDI). The SDI represents how near to a perfect circle a lake's shoreline is. Lakes with higher SDI values would contain more bays and side channels, likely creating more littoral zone habitat. However, SDI was not a significant predictor of *L. stagnalis* presence. Thus, this relationship remains unknown, but suggests lake morphology is important for *L. stagnalis*' success in a habitat. Sample size is small (N = 11), which may confound this relationship and SDI values varied widely (mean ± s.d. = 2.56 ± 1.96).

Parasitism

Trematode cercariae were found sporadically across northeastern Minnesota. *L. megasoma* was the primary source of trematode cercariae on the landscape. Trematode infection shortened the life of *L. megasoma* in captivity, likely through tissue damage and energy depletion associated with parasitic infections (Sorensen and Minchella 2001). The consequences of a truncated *L. megasoma* lifespan are not known. Parasitic infection in some snails has been related to ecological stoichiometry and may represent an important part of ecosystem nutrient cycling and function (Bernot 2013). The size, longevity, ease of laboratory maintenance, and ubiquity of *L. megasoma* in northern Minnesota, make this species an excellent candidate for further parasitological study. However, little is known about this species ecologically or as a parasite intermediate host (Clarke 1973, 1981, Gilbertson et al. 1978, Laursen et al. 1992, McKillop et al. 1993). Our study suggests that *L. megasoma* may be the primary host of many trematode parasites in northern Minnesota since 14 of 16 trematode infected snails were *L. megasoma*.

The data presented in this study lays the groundwork for further habitat modelling of *L. megasoma* and *L. stagnalis*. Ordination techniques and indicator species analysis show great promise as supplements to traditional modelling procedures and may inform trematode infection risk analyses in the future.

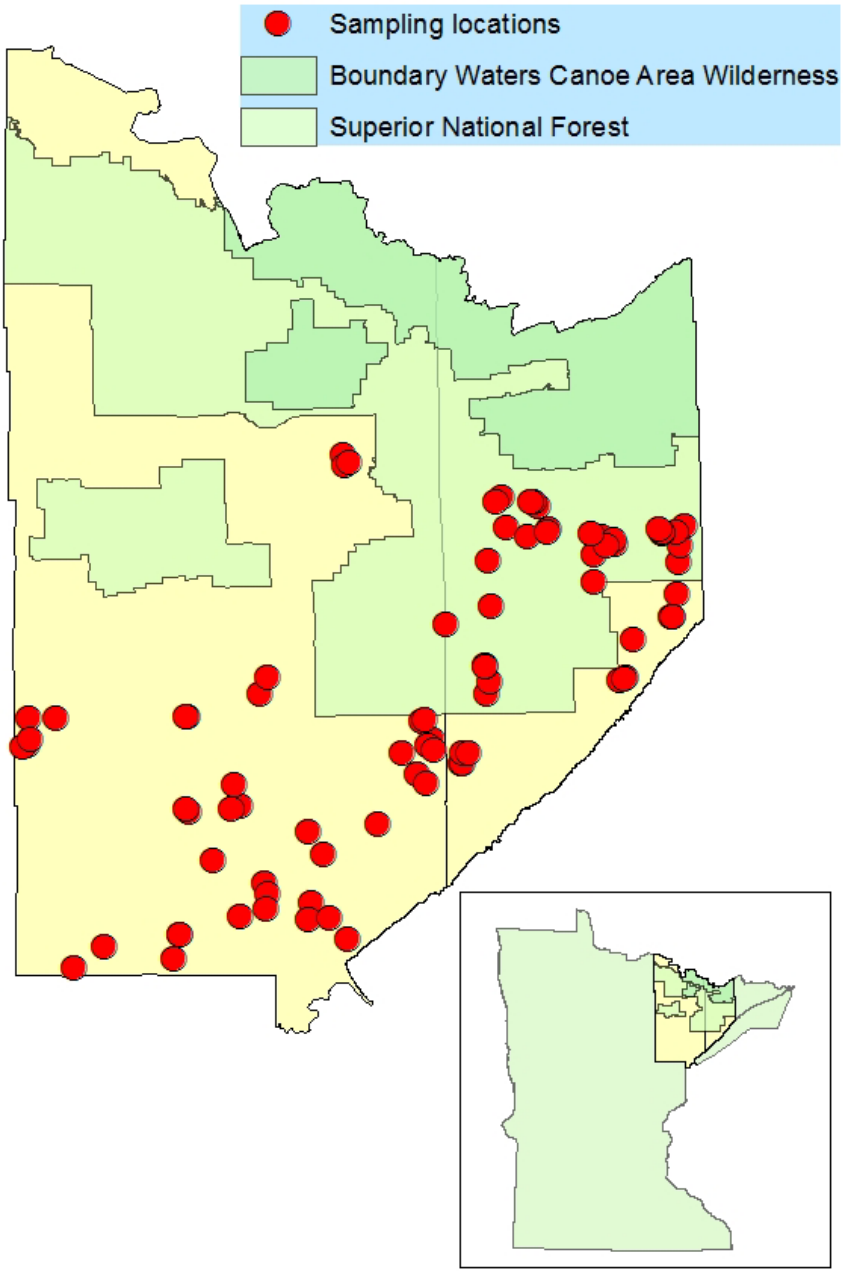


Figure 1. Lymnaeid snail sampling sites in St. Louis County and Lake County, Minnesota, USA. (N = 84).

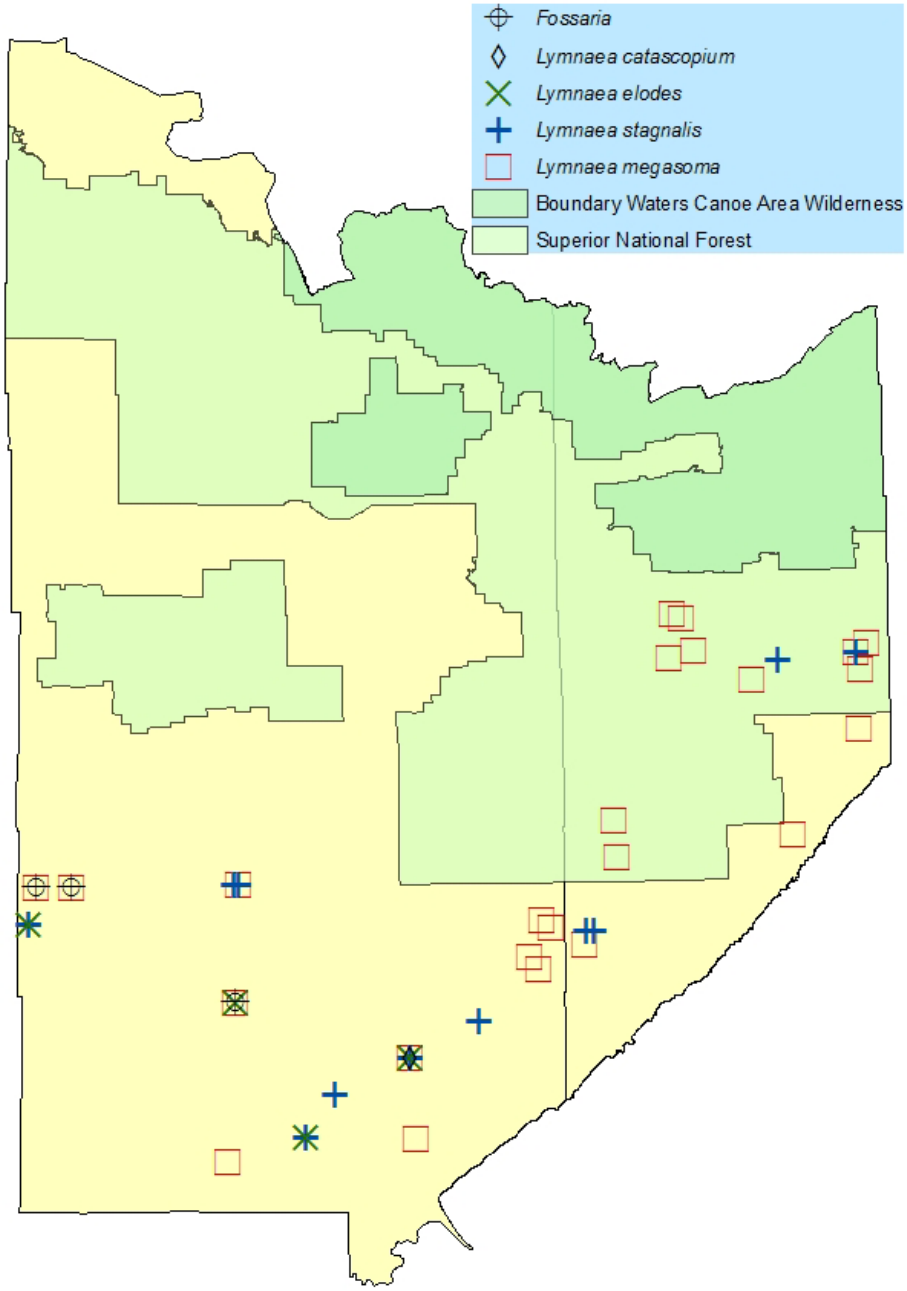


Figure 2. Lymnaeid snail distributions in St. Louis County and Lake County, Minnesota, USA.

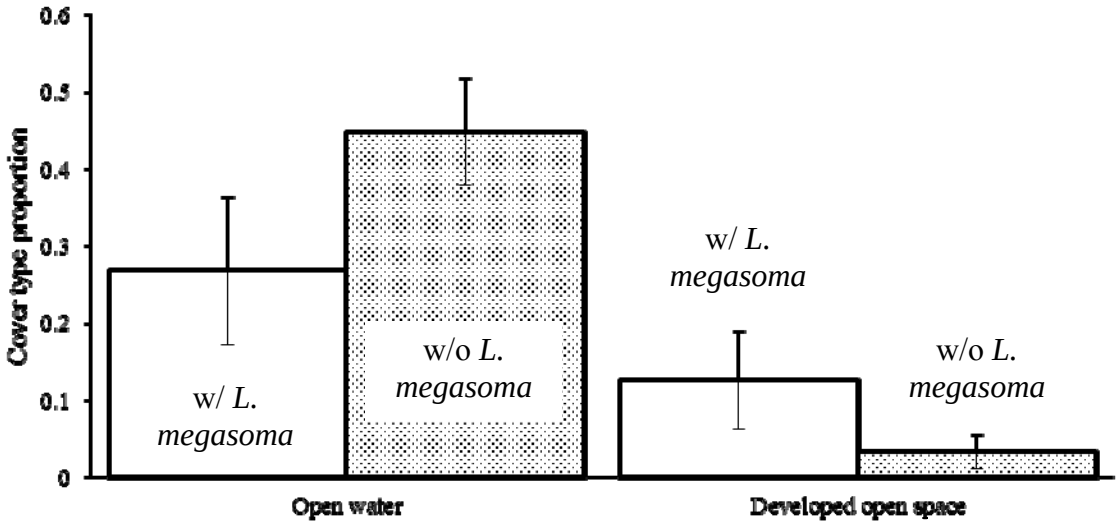


Figure 3. Proportions of NLCD open water and developed open space within a 100 m buffer in areas with (hollow) and without (speckled) *L. megasoma*. Open water mean with *L. megasoma* = 0.27, without = 0.45, two sample Z test, $Z_{82} = 2.96$, p = 0.002; Developed open space mean with *L. megasoma* = 0.13, without = 0.03, $Z_{82} = -2.69$, p = 0.004. Error bars represent 95% confidence intervals.

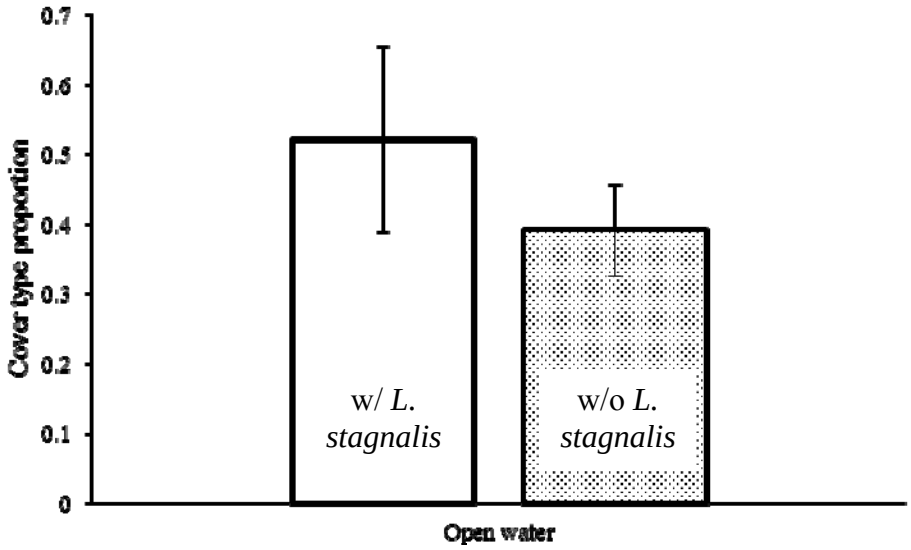


Figure 4. Proportion of LULC open water in areas with (hollow) and without (speckled) *L. stagnalis*. Open water mean with *L. stagnalis* = 0.52, without = 0.39, $Z_{82} = -1.73$, p = 0.04. Error bars represent 95% confidence intervals.

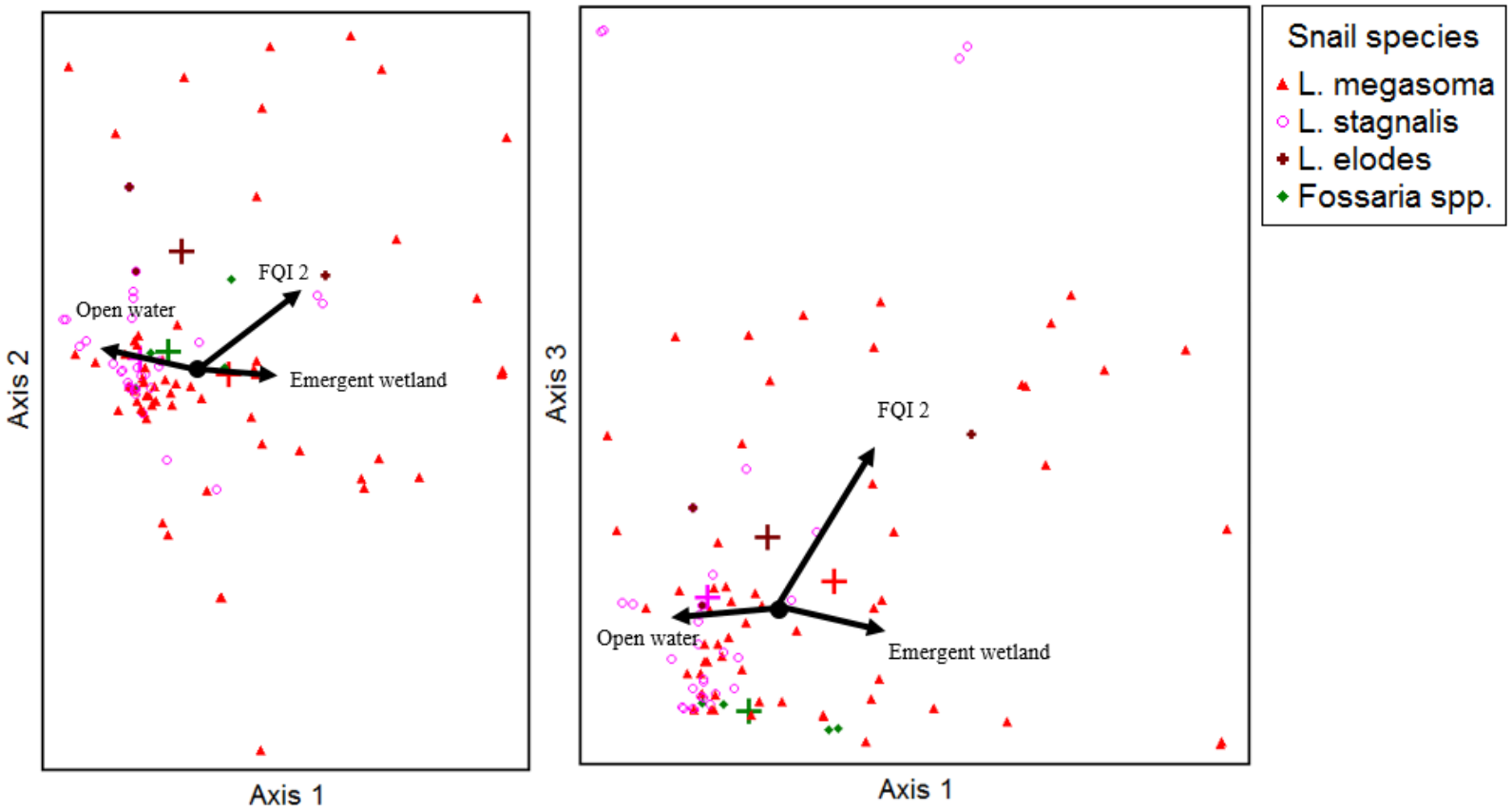
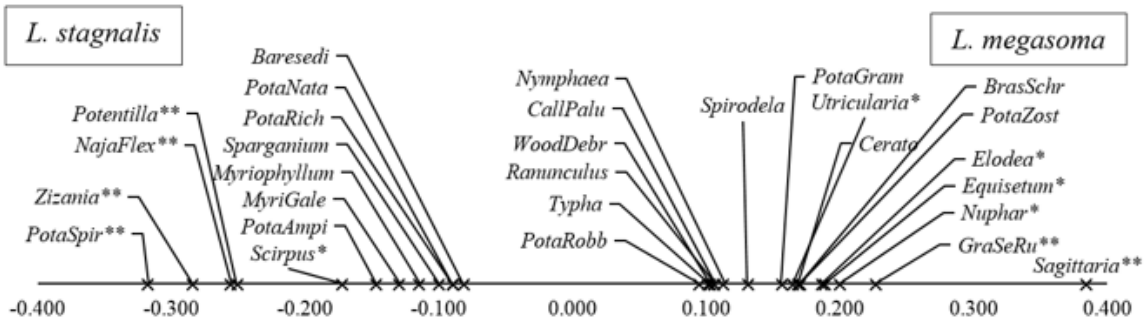


Figure 5. Nonmetric multidimensional scaling plot with a joint plot ($r^2 \geq 0.20$ for inclusion) radiating from the centroid. FQI, proportion of open water, and proportion of emergent wetland on the landscape were important environmental variables. Snail vegetation quadrats clustered near the centroid due to bare sediment being common within vegetation plots.

Table 1. Multi-response permutation procedures output for snail species groupings. All pairwise comparisons show there is a difference between the groupings (*Fossaria* spp., N = 33 quadrats; *Lymnaea megasoma*, N = 89 quadrats; *L. stagnalis*, N = 76 quadrats; *L. elodes*, N = 4 quadrats). *A* represents the chance-corrected within-group agreement (Biondini et al. 1985, McCune et al. 2002).

Species groups	*A*	P value
Fossaria* v. *L. megasoma	0.069	0.00
Fossaria* v. *L. stagnalis	0.063	0.00
Fossaria* v. *L. elodes	0.144	0.00
L. megasoma* v. *L. stagnalis	0.040	0.00
L. megasoma* v. *L. elodes	0.017	0.01
L. stagnalis* v. *L. elodes	0.023	0.02



Vegetation species	Group	Observed Indicator Value	Mean	Sdev	P value
Sagittaria spp.**		0.385	0.064	0.046	< 0.01
Emergent grass, sedge, or rush**		0.227	0.062	0.048	< 0.01
Nuphar spp.*		0.200	0.064	0.047	0.01
Equisetum spp.*		0.189	0.065	0.045	0.02
Elodea spp.*		0.187	0.063	0.046	0.03
Potamogeton zosterformis		0.170	0.065	0.044	0.06
Brasenia schreberi		0.170	0.065	0.044	0.06
Ceratophyllum spp.	*L. megasoma*	0.169	0.062	0.048	0.05
Utricularia spp.*		0.165	0.063	0.048	0.04
Potamogeton gramineus		0.156	0.064	0.045	0.07
Spirodela spp.		0.131	0.067	0.04	0.25
Nymphaea spp.		0.114	0.063	0.047	0.17
Calla palustris		0.107	0.06	0.051	0.50
Ranunculus spp.		0.107	0.061	0.051	0.51
Woody debris		0.103	0.063	0.046	0.23
Typha spp.		0.102	0.063	0.049	0.24
Potamogeton robbinsii		0.095	0.065	0.044	0.38
Bare sediment		-0.082	0.062	0.048	0.41
Potamogeton richardsonii		-0.090	0.064	0.047	0.34
Potamogeton natans		-0.090	0.063	0.047	0.33
Sparganium spp.		-0.100	0.063	0.048	0.26
Myriophyllum spp.		-0.114	0.063	0.045	0.22
Myrica gale	*L. stagnalis*	-0.129	0.063	0.048	0.10
Potamogeton ampifolius		-0.147	0.064	0.047	0.07
Scirpus spp.*		-0.172	0.061	0.047	0.04
*Potentilla palustris***		-0.251	0.063	0.047	< 0.01
*Najas flexilis***		-0.255	0.062	0.047	< 0.01
Zizania spp.**		-0.284	0.062	0.047	< 0.01
*Potamogeton spirillus***		-0.317	0.064	0.047	< 0.01

Figure 6. Graph and table of observed indicator values for quadrat data. Numbers approaching -1 are stronger indicators of an *L. stagnalis* site. Numbers approaching 1 indicate *L. megasoma.*

Figure 6 cont. Key to species codes for indicator species analysis.

Abbreviation	Vegetation species	Group
Sagittaria	***Sagittaria* spp.****	
GraSeRu	**Emergent grass, sedge, or rush****	
Nuphar	***Nuphar* spp.***	
Equisetum	***Equisetum* spp.***	
Elodea	***Elodea* spp.***	
PotaZost	***Potamogeton zosterformis***	
BrasSchr	***Brasenia schreberi***	
Cerato	***Ceratophyllum* spp.**	*L. megasoma*
Utricularia	***Utricularia* spp.***	
PotaGram	***Potamogeton gramineus***	
Spirodela	***Spirodela* spp.**	
Nymphaea	***Nymphaea* spp.**	
CallPalu	***Calla palustris***	
Ranunculus	***Ranunculus* spp.**	
WoodDebr	**Woody debris**	
Typha	***Typha* spp.**	
PotaRobb	***Potamogeton robbinsii***	
Baresedi	**Bare sediment**	
PotaRich	***Potamogeton richardsonii***	
PotaNata	***Potamogeton natans***	
Sparganium	***Sparganium* spp.**	
Myriophyllum	***Myriophyllum* spp.**	
MyriGale	***Myrica gale***	*L. stagnalis*
PotaAmpi	***Potamogeton ampifolius***	
Scirpus	***Scirpus* spp.***	
Potentilla	***Potentilla palustris****	
NajaFlex	***Najas flexilis****	
Zizania	***Zizania* spp.****	
PotaSpir	***Potamogeton spirillus****	

Table 2. Univariate logistic regression models for *Lymnaea megasoma* and *L. stagnalis* presence/absence data using various environmental variables.

ΔAIC	Akaike weight	Covariate	Coefficient	p-value	Hosmer-Lemeshow p-value
L. megasoma					
0	0.472	Open water	-3.10	< 0.01	0.10
0.24	0.418	[$CaCO_3$]	0.025	0.01	0.40
4.5	0.050	Average depth	-0.12	0.04	0.27
5.2	0.035	Lake fetch	-0.00014	0.07	0.45
6.7	0.017	DCA Ratio	0.021	0.13	0.84
7.8	0.009	Intercept only	-0.83	< 0.01	na
-0.253	*na*	*Developed open space*	*7.00*	*< 0.01*	*1*
L. stagnalis					
0	0.602	Width : Length Ratio	4.85	0.02	0.48
2.6	0.164	Littoral Area	0.0011	0.06	0.38
3.7	0.095	SDI	0.41	0.08	0.22
4.0	0.081	Lake Area	0.00046	0.10	0.29
4.7	0.057	Intercept only	-1.89	< 0.01	na
1.0	*na*	*Mixed forest*	*6.47*	*0.02*	*1*

Table 3. Univariate logistic regression models for *L. megasoma* and *L. stagnalis* presence/absence data using FQI_1 and FQI_2. Data consisted of N = 92 *L. megasoma* quadrats, N = 77 *L. stagnalis* quadrats, and N = 62 random quadrats.

Species	Covariate	ΔAIC	Coefficient	p-value	Hosmer-Lemeshow p-value
L. megasoma	FQI_1	0	-0.13	< 0.01	0.14
	FQI_2	7.36	-0.012	0.06	0.65
L. stagnalis	FQI_1	8.93	-0.076	0.06	< 0.01
	FQI_2	0	-0.026	< 0.01	0.02

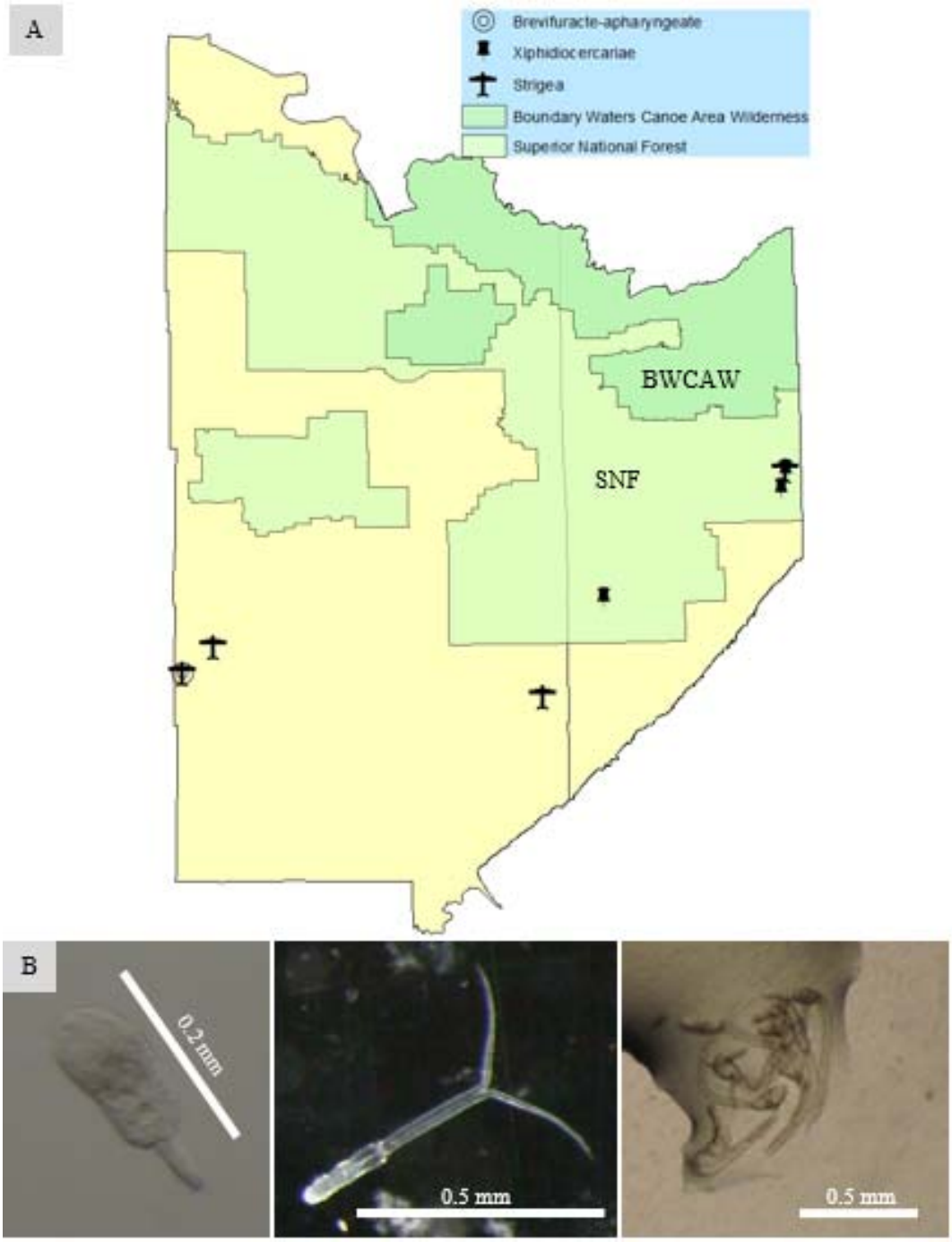


Figure 7. (A) Locations where cercariae were found. (B) Images of trematode cercariae. From left to right, Xiphidiocercariae (likely Armatae), Strigea, and brevifurcate-apharyngeate.

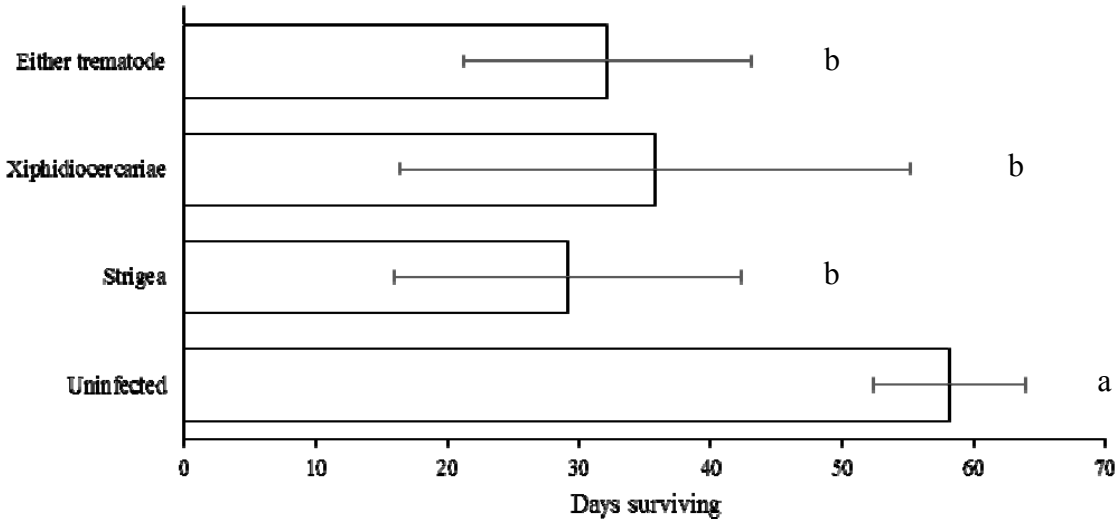


Figure 8. Mean number of days surviving for *L. megasoma* uninfected and infected with certain trematodes. Uninfected snails survived longer than all other groups. Error bars represent 95% confidence intervals.

Table 4. Sampling effort of similar snail distribution and habitat studies near Minnesota or with a similar target group.

Number of Water Bodies	Number of Samples / Water Body	Time / Water Body	Number of Field Seasons	Target Group	State / Province	Source
72	1-3	~1 hr	1	Lymnaeid snails	Minnesota	Current study
25	1-13	na	2	Lymnaeid snails	Minnesota	(Laursen 1993)
148	na	na	1	Pulmonate snails	Minnesota	(Laursen et al. 1992)
270	1-3	na	3	Molluscs	Minnesota	(Dawley 1947)
18	1-7	na	1	Molluscs	Manitoba	(Pip 1992)
90	1	~1 hr	1	Molluscs	Manitoba	(Pip 2006)
308	1	~1 hr	1	Molluscs	Manitoba	(Pip 2000)
319	1	~1 hr	4	Molluscs	Manitoba	(Pip 2000)
27	na	na	2	Gastropods	Michigan	(Archer 1939)
37	7	na	7	Gastropods	Michigan	(Hoverman et al. 2011)
48	na	na	2	Molluscs and other fauna	Michigan	(Adams 1909)
97	na	na	3	Lymnaeid snails	Montana	(Dunkel et al. 1996)
13	na	~3 hr	1	Molluscs	North Dakota	(Tuthill and Laird 1963)
90	10 dips w/ dipnet	na	4	Lymnaeid snails	North Dakota	(Maskey 2011)
298	35 sampled twice	0.5-2 hr	9	Molluscs	North Dakota	(Cvancara 1983)
25	14	na	2	Pulmonate snails	Ontario	(McKillop and Harrison 1972)
6	37	na	2	Pulmonate snails	Wisconsin	(Colburn 1975)
18	~12	na	3	Gastropods	Wisconsin	(Lewis and Magnuson 2000)

na = metric was not explicitly stated

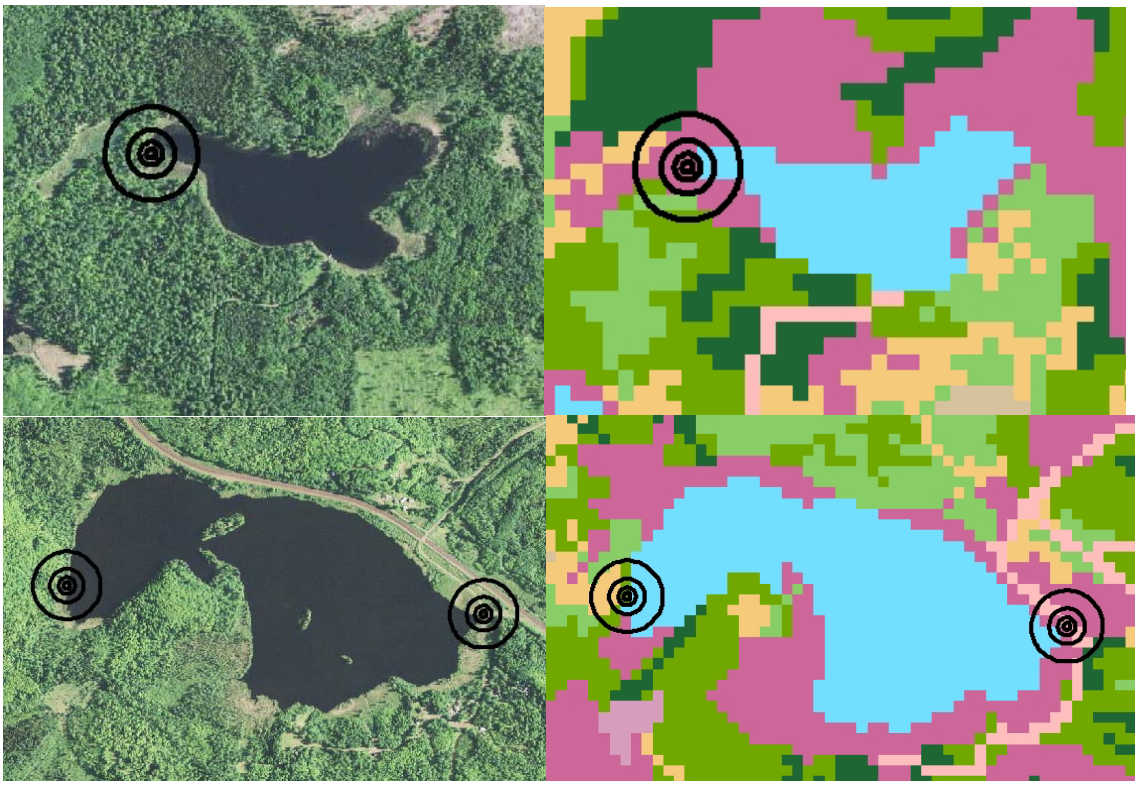


Figure 9. Farm Service Administration aerial imagery from 2013 and NLCD data for (top) one *L. megasoma* site and (bottom) two *L. stagnalis* sites. Black circles represent 10, 25, 50, and 100 m buffer zones around sampling sites. More open water is apparent within the *L. stagnalis* buffer zones compared to *L. megasoma*.

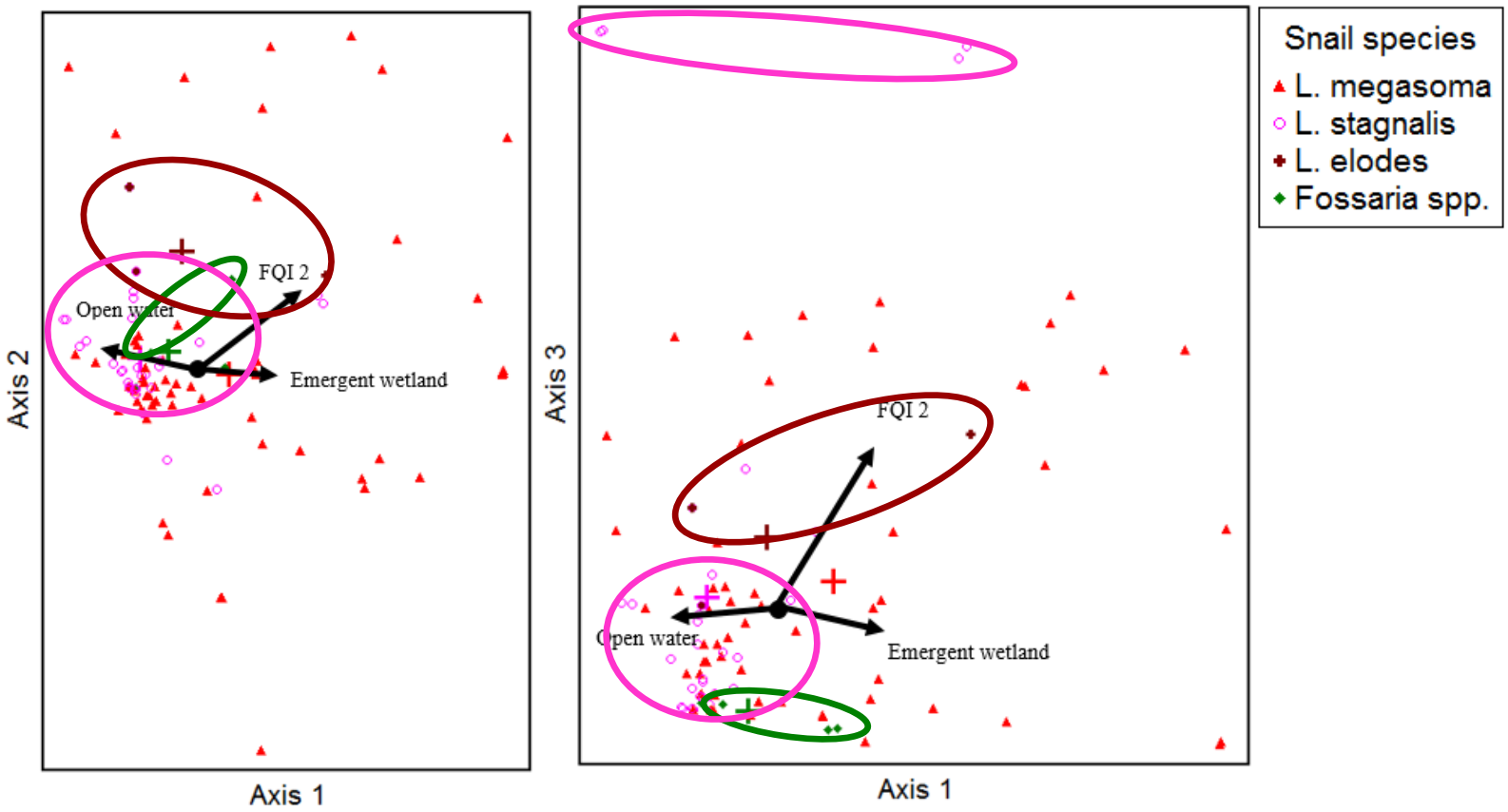


Figure 10. NMS plot with species groupings circled. Two *L. stagnalis* ecotypes may be present. No circle is drawn around *L. megasoma*, which populates most of the plot.

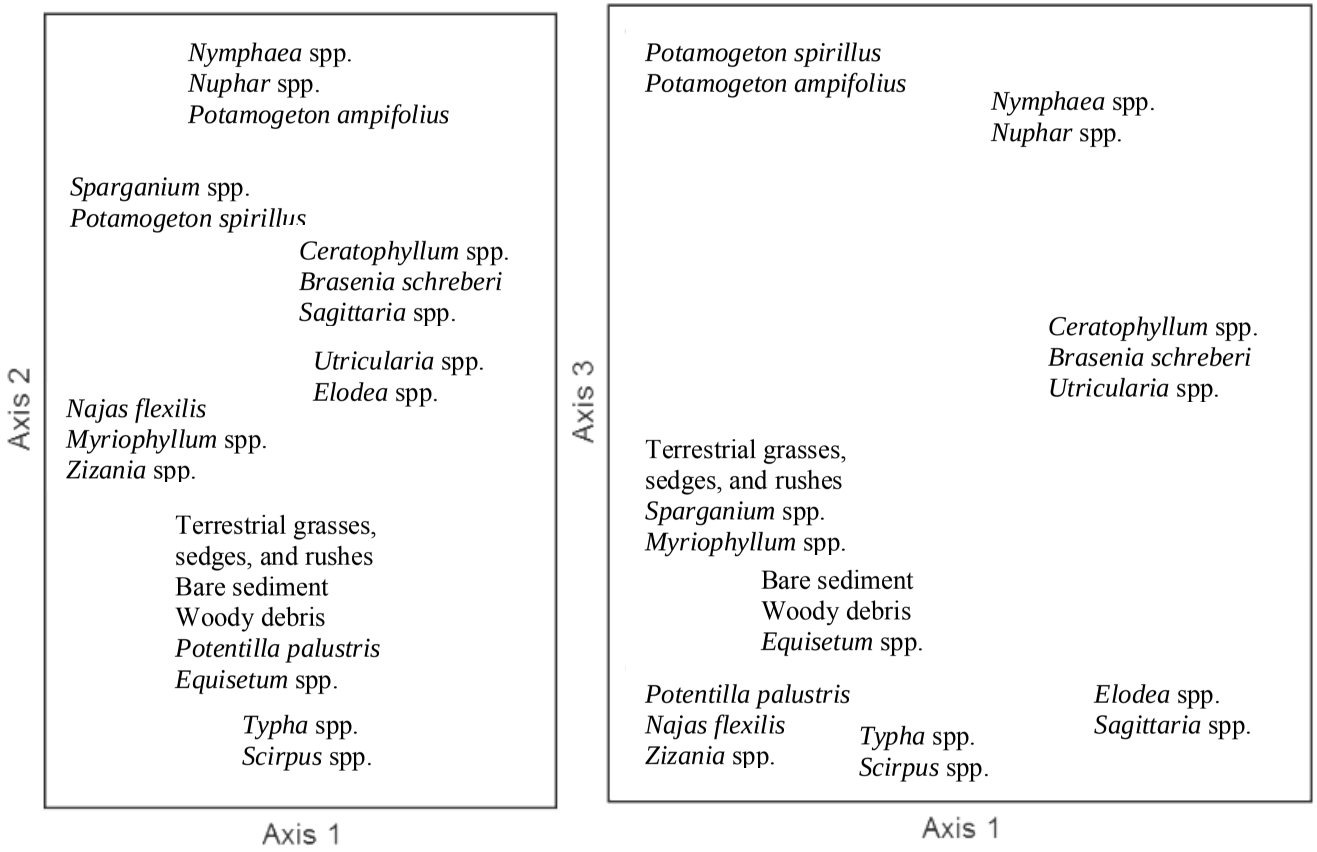


Figure 11. Representative locations for certain plant communities in species space.

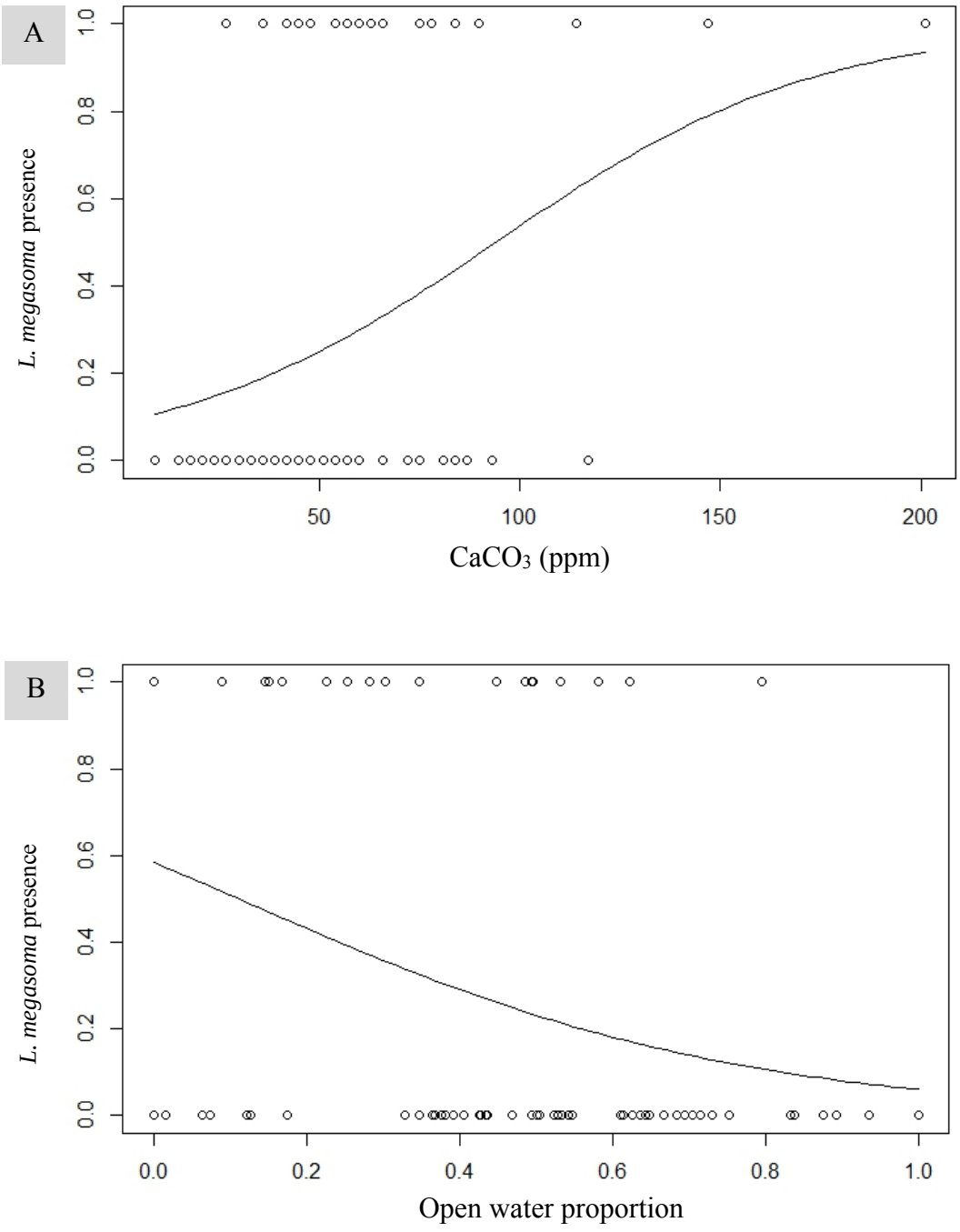


Figure 12. Univariate logistic regression models of (A) calcium carbonate (ppm) and (B) open water proportion against *L. megasoma* presence/absence. See Table 2 for statistics.

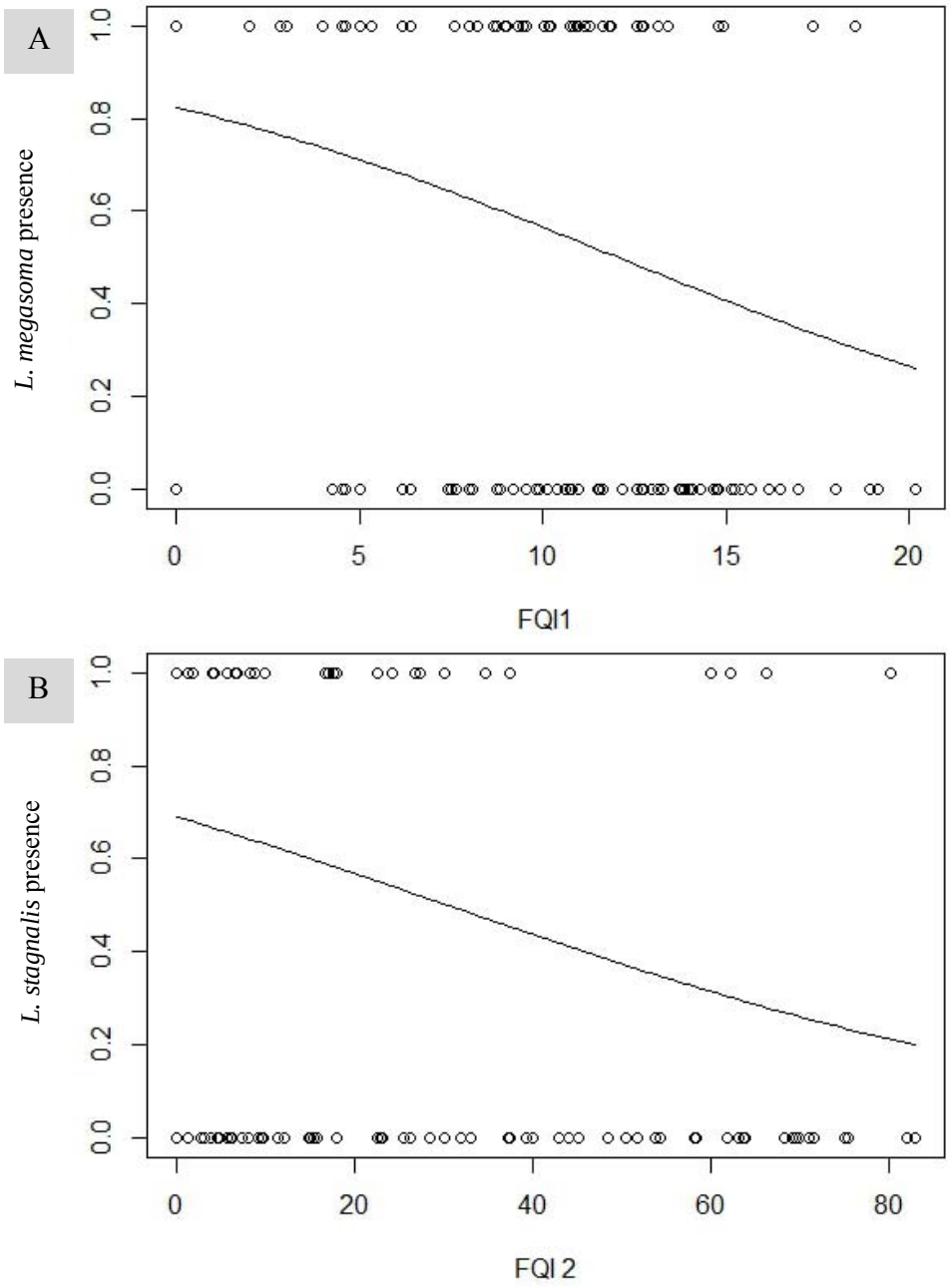


Figure 13. (A) Generalized linear model of FQI_1 values against *L. megasoma* presence/absence. FQI_1 was a significant explanatory variable of snail presence ($p < 0.01$) using the logistic model logit (π_{FQI1}) = 1.54 + -0.13(FQI_1). Modelled data was not significantly different from the observed data (Hosmer-Lemeshow goodness of fit test, $p = 0.14$). (B) Generalized linear model of FQI_2 against *L. stagnalis* presence/absence. FQI_2 was a significant explanatory variable of *L. stagnalis* presence ($p < 0.01$) using the logistic model logit (π_{FQI2}) = 0.80 + -0.026 (FQI_2). Modelled data was significantly different from the observed data (Hosmer-Lemeshow goodness of fit test, $p = 0.02$).

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Chapter 3:

Giant Liver Fluke and Thin-necked Bladderworm from Hunter-harvested White-tailed Deer: Prevalence, Coinfection, Liver Habitat, and Landscape Patterns

Summary

The giant liver fluke and thin-necked bladderworm are parasites of unknown significance for white-tailed deer. However, the prevalence of giant liver fluke is increasing in many locations across North America. Between the winters of 2014 and 2015, 125 white-tailed deer livers were collected from the city of Duluth, Minnesota, USA. Prevalence of giant liver fluke was 42% and prevalence of thin-necked bladderworm was 18%. Logistic regression showed the probability of infection was explained partially by liver weight for both species, likely associated with host age. Coinfection with both parasites was also common (14%) and a statistically significant association between infections was found. This association may have been related to abiotic factors, deer behavior, or immunology. Specific liver regions are speculated to be preferred habitat for fluke encapsulation. Fluke biomass in deer livers correlated strongly with the area of emergent herbaceous wetlands and woody wetlands in deer hunting areas. Bladderworm infection metrics were not strongly correlated with any landscape variable.

Introduction

The giant liver fluke (*Fascioloides magna*) is a trematode parasite which infects white-tailed deer and other cervids in North America (Pybus 2001). Deer are often infected with giant liver flukes and many deer are also coinfected with a cestode parasite called the thin-necked bladderworm (*Taenia hydatigena*) (Addison et al. 1988). The prevalence of liver flukes in North America appears to be increasing (Vannatta and Moen *in review*, Pybus et al. 2015). However, the effects of liver flukes and bladderworms on deer

mortality are not fully known. Some authors have suggested liver flukes may contribute to mortality in cervids (Cheatum 1951, Murray et al. 2006), while others have documented heavy fluke burdens with no apparent effects (Pursglove et al. 1977, Lankester and Foreyt 2011). Bladderworms also appear largely innocuous (Jones and Pybus 2001) except in extreme cases (Gregson 1937). However, sublethal fitness effects of liver fluke infections in white-tailed deer have been documented (Mulvey and Aho 1993, Mulvey et al. 1994).

Most studies examine the biology of parasites in single-host, single-parasite systems. However, parasites often persist in a diverse host environment with many individuals and species involved in various within-host symbioses (Telfer et al. 2010). These coinfections can vary greatly (Telfer et al. 2010). In some circumstances, one infection may facilitate establishment, hinder establishment, lessen the pathology, or exacerbate the pathology of another parasite (Telfer et al. 2010, Johnson and Buller 2011, Johnson and Hoverman 2012, Garza-Cuartero et al. 2014). Tractable systems for studying the impacts of coinfection in wildlife are needed.

Additionally, a number of giant liver fluke studies have examined the effects of landscape level variables on disease (Mulvey et al. 1994, Vanderwaal et al. 2014, Pybus et al. 2015). However, with the exception of VanderWaal et al., these studies used very coarse landscape level variables and VanderWaal et al. used deer pellets to diagnose infection, which can be unreliable (Boray 1969, Erhardova-Kotrla 1971, Blazek et al. 1972). The effects of landscape level variables on bladderworm infection is also not known. Determining landscape level risk factors for infection is increasingly important as

climate change will impact complex life cycles parasites and change landscapes (Pickles et al. 2013).

The increasing presence and density of deer in towns and cities over recent decades gives scientists unprecedented access for monitoring wildlife disease in the urban mosaic. The city of Duluth, Minnesota, USA is an ideal location for this type of study because a bowhunt within city limits harvests >400 deer annually, and cover type composition varies within the city. The current study seeks to document a baseline level of infection for liver flukes and bladderworms within an urban environment and lay a foundation for long term monitoring of disease dynamics within these landscapes.

Methods

Bowhunters collected deer livers from September to December of 2014 and 2015 in deer hunting areas (DHAs) in the city of Duluth, Minnesota, USA (Fig. 1A). Livers were frozen up to five months before processing. After thawing, livers were weighed, and volume was measured by water displacement. Livers were then sectioned into 0.5 to 1.5 cm slices and examined for fluke capsules and bladderworm cysts. The number of bladderworms, mature flukes, fluke capsules, and flukes per capsule was recorded for each liver. Additionally, fluke length, width, and wet mass was recorded. In 2015, the location of each fluke capsule within the liver was noted (Fig. 2A-D).

For landscape analyses, DHA polygons were drawn in ArcMap (v10.3, ESRI Inc., Redlands, CA). DHAs in which deer were harvested in 2015 were merged into five subregions for further analysis (Fig. 1B). The proportion of land cover within each

harvest subregion was determined using the National Land Cover Dataset (NLCD; U.S. Geological survey) from 2011 and the Geospatial Modelling Environment (v0.7.4.0, Dr. Hawthorne L. Beyer, Spatial Ecology LLC). Open water, emergent herbaceous wetlands, and woody wetlands were combined into a 'wet cover type' class. All other land cover types were analyzed individually. Landscape correlations with various measures of liver fluke and bladderworm infection (Table 1) were only evaluated if R^2 was greater than 0.60.

Results

In 2014 and 2015 125 deer livers were examined (2014, N = 49; 2015, N = 76). The prevalence of liver flukes and bladderworms was 42% and 18%, respectively. Prevalence did not vary between years for liver flukes (35% in 2014; 46% in 2015, two proportion Z test, $Z_{123} = 1.26$, $p = 0.208$) or bladderworms (12% in 2014; 22% in 2015, $Z_{123} = 1.43$ $p = 0.154$). Data from both years were combined for all subsequent analyses.

Liver weight was significantly higher in deer parasitized by liver flukes (mean ± s.d. = 1,302 ± 271 g) than deer not parasitized by liver flukes (mean ± s.d. = 974 ± 308 g; one tailed Z test: $Z_{123} = -6.3$, $p << 0.0001$). This trend was also present in bladderworm infections (mean parasitized liver weight ± s.d. = 1,263 ± 252 g, mean unparasitized liver weight ± s.d. = 1,076 ± 341 g; $Z_{123} = -3.0$, $p = 0.001$). Generalized linear models (GLM) of liver fluke (Fig. 3A) and bladderworm presence/absence (Fig. 3B) yielded liver weight as a significant explanatory variable. Deer coinfected with liver flukes and bladderworms did not have higher liver weights than deer infected with only one parasite ($Z_{123} = 1.15$, p

= 0.13). Liver weight was not a significant explanatory variable of coinfection versus single infection ($p = 0.31$) and did not decrease model deviance ($p = 0.31$).

Coinfection with liver flukes and bladderworms occurred in 14% of deer. Of 58 infections, 6 infections only involved bladderworms, 35 involved only liver flukes, and 17 were coinfections. A χ^2 test of the fluke x bladderworm contingency table suggests the two infections are associated with one another ($\chi^2 = 12.11$, $df = 1$, $p = 0.0005$). The proportion of deer with bladderworm infections that also had liver fluke infections was greater than the proportion of deer without bladderworms having fluke infections (74% compared to 34%; one tailed two proportion Z test: $Z_{123} = -3.48$, $p = 0.0003$).

Most liver fluke infections had few flukes with 1-6 flukes present in 77% of infections (Fig. 4). However, the remaining infected animals had 7-15 flukes, which accounted for 52% of the total recovered flukes. This equates to 52% of the sampled fluke population present within 10% of the harvested deer. Bladderworms also appear aggregated with 35% of bladderworms carried by 17% of infected deer or 3% of the harvested deer (Fig. 5).

Liver flukes were most often found in pairs within liver capsules (Fig. 6). Capsules were primarily found in the right lobe of the liver in the center of the liver parenchyma (Fig. 2). Although less common, capsules were also found in the left lobe, but flukes were rarely found within the caudate lobe. Capsules were common in the central parenchyma, but less common in the anterior and posterior portions of the liver. These trends were largely mirrored by the number of flukes within each region (Fig. 2).

However, higher proportions of flukes were found in the right-center liver region compared to the proportion of capsules.

The numbers of fluke capsules, flukes, and bladderworm cysticerci were not strongly correlated with liver weight (see Table 2 for regression summaries not presented in other figures). Additionally, fluke length, width, and mass were not correlated with liver weight. These measures were not compared with liver volume as liver weight and volume were correlated.

The proportion of cover types varied in each DHA subregion (Table 3 and Fig. 7). Liver weight was greater in developed open spaces and less in conifer ('Evergreen') forest area. However, since liver weight was not strongly correlated with the number of flukes, fluke mass, or bladderworm cysts in deer livers, these correlations did not likely confound other measures of infection. Fluke prevalence increased with the proportion of low to moderate developed space and shrub/scrub cover types, and decreased with area of deciduous forest cover types in DHA subregions. Total fluke mass incerased with the proportion of wet cover types in each DHA (Fig. 8). Wet cover types consisted of a combination of open water, woody wetlands, and emergent herbaceous wetlands. When these contributing cover type classes were separated, emergent herbaceous wetlands and woody wetlands were highly correlated with total fluke mass despite these cover types being rare (Fig. 9 and Table 3). Open water habitat did not correlate with fluke mass.

Bladderworm infection measurements had few significant landscape correlations (Table 2). The number of deer infected with bladderworm was loosely and positively correlated with mixed forest cover types.

Discussion

Many attributes of liver fluke and bladderworm parasitism documented here have been seen in other parasite populations. The liver fluke prevalence in this study (42%) was not significantly different from a similar study in Ontario, Canada (two proportion Z test, $Z_{282} = -1.64$, $p = 0.10$; Addison et al. 1988). However, fluke prevalence appears to have increased in Minnesota by ~20% since the1940s (Olsen and Fenstermacher 1943). Bladderworm prevalence has seen little change over this time period, only increasing from 14% to 18% (Olsen and Fenstermacher 1943). Although multiple factors likely contribute to the prevalence of these parasites, the range expansion and rising density of white-tailed deer in northern climates (Hewitt 2011) is likely an important factor.

The correlation between parasite prevalence and liver weight is likely a result of deer age. Various studies have documented increasing infection prevalence and sometimes increasing intensity of infection with age for liver flukes (Cheatum 1951, Foreyt et al. 1977, Addison et al. 1988, Pybus 1990, Laursen 1993, Mulvey and Aho 1993, Qureshi et al. 1994, reviewed by Vannatta and Moen *in review*) and bladderworms (Torgerson et al. 1995). This suggests both parasite infections are acquired over time and are likely related to certain feeding behaviors, which may give rise to coinfection.

Coinfection with these parasites was common with 14% of harvested deer containing both infections. The intensity of infections were not correlated ($r^2 = 0.063$). Factors likely explaining these coinfections include: abiotic factors affecting parasite viability, individual behavioral differences in deer, and variation in immune response to infection. The abiotic factors limiting parasite viability are similar between the two organisms. The life cycle of the giant liver fluke requires infection of a snail intermediate host and subsequent encystment on aquatic vegetation (Pybus 2001). As such, fluke larval stages are highly dependent on temperature and humidity (Griffiths and Christensen 1972, 1974, Pybus 2001). Similar abiotic restrictions have been outlined for bladderworms (Laws 1968, Gemmell and Johnstone 1976, Coman and Rickard 1977, Gemmell 1977, Gemmell et al. 1987, Torgerson et al. 1995). Consequently, locations with suitable temperature and humidity could simply extend the viability of fluke and bladderworm larval stages, making these areas a higher risk for parasitic infection. However, the lack of landscape correlates with bladderworm infection suggests deer behavior or immunology is driving coinfection.

Since both bladderworm and fluke larval stages must be ingested by deer for infection to occur, individual deer foraging behavior likely plays a key role in coinfection. Deer feeding behavior near aquatic habitats is not well documented or understood (Hennings 1977, Ceacero et al. 2014, Vannatta and Moen *in review*), but fluke infection is most likely acquired when deer feed near vernal pools in spring and fall on the first and last green vegetation (Vannatta and Moen *in review*). Bladderworms infect canids as definitive hosts and deer as intermediate hosts and thus are not coupled to

aquatic environments (Jones and Pybus 2001). Deer could potentially become infected feeding in any location near infected canid scat. However, the effects of temperature and humidity on bladderworm viability outlined above suggests that deer feeding in damp areas near vernal pools could lead to coinfection.

Outside of deer feeding behavior, individual immune variation could leave certain individuals more prone to infection. Alternatively, the establishment of one parasite could modulate the immune response of the host, making subsequent infections more easily acquired (Maizels et al. 1993). Parasites closely related to giant liver fluke (e.g. *Fasciola hepatica*) and thin-necked bladderworm (e.g. *Taenia solium* and *T. taeniaeformis*) are known to alter host immune responses (Maizels et al. 1993) possibly facilitating establishment of other parasite species. However, a lack of relationship between fluke and bladderworm abundance within a host suggests immune modulation may lower a colonization threshold, but not be a strong indicator of abundance ($r^2 = 0.063$). It should be noted that coinfection with liver flukes and bladderworms does not suggest that these two species are mutualists or commensals. These parasites may be antagonistic toward each other (Telfer et al. 2010, Garza-Cuartero et al. 2014). More work needs to be done to determine if coinfection exacerbates fitness costs in deer or lessens the impact of these parasite infections (Garza-Cuartero et al. 2014).

The pairing of flukes within liver capsules was quite common (50% of capsules contained two flukes; Fig. 6). This pairing tendency has been described previously (Foreyt et al. 1977). However, the flukes in this study only paired in 50% of capsules compared to 85% in Foreyt et al.'s study (1977). A high incidence of single fluke

capsules was reported from the current study. These capsules were often underdeveloped with very thin walls, but a lack of liver hemorrhage around these flukes suggests they were indeed capsules and not migratory tracks.

Our study suggests the right lobe of the liver and specifically the right-central lobe of the liver may be the best location for fluke encapsulation (Fig. 2). Capsules in this area were often in close proximity to major blood vessels (Fig. 10). It is possible that this location may yield a constant and abundant supply of oxygen and other nutrients. However, the right lobe of the liver is larger than the left and caudate lobes. Whether these trends are a result of habitat selection by flukes or a result of simple probability is not known and warrants further consideration.

Few studies have looked for landscape level correlates of liver fluke infection (Mulvey et al. 1994, Vanderwaal et al. 2014, Pybus et al. 2015), but the increasing accuracy and utility of landscape level tools has made these analyses accessible. Previous work compared upland and swamp locations finding that fluke prevalence was higher in swamp areas (Mulvey et al. 1994). Another habitat type called rooted-floating aquatic marshes was a significant predictor of fluke infections in Voyageurs National Park in Minnesota (Vanderwaal et al. 2014). This was likely related to palatable aquatic forage. In the current study, we assumed fluke infection was acquired within the DHA subregion where the deer was harvested. This assumption is not unreasonable as urban deer home ranges are often 40 - 60 hectares and dispersal distances are less than 9 km (Hewitt 2011), well within the range of the DHA subregion areas (South ≈ 1670 ha, South Central ≈ 880 ha, Central ≈ 370, Campus ≈ 1820 ha, North ≈ 1750).

Fluke biomass was strongly correlated with wet cover types, specifically, emergent herbaceous wetlands and woody wetlands (Fig. 8 and 9), despite these habitats contributing a small proportion of DHA area (Table 3). These correlations are possibly related to snail intermediate host habitat. The primary host of giant liver flukes are snails in the *Fossaria* genus (Swales 1935, Laursen and Stromberg 1993). These snails are residents of ephemeral water habitats and vernal pools (McCraw 1959, 1961, Clarke 1981, Laursen et al. 1992, Laursen and Stromberg 1993, Dunkel et al. 1996). It is possible that this explains the lack of correlation between open water, which would not make ideal *Fossaria* spp. habitat, and fluke biomass. The remaining correlations between land cover and fluke prevalence may be explained by *Fossaria* spp. being uncommon in upland forests and often found in roadside ditches (Laursen et al. 1992).

Although landscape level variables were consistent with previous work and current knowledge regarding liver flukes, bladderworm infection measures did not correlate well with any cover types. This is not surprising as both the definitive bladderworm host (canids) and the intermediate host (white-tailed deer) are highly mobile and not restricted to certain habitat types (Jones and Pybus 2001). Additionally, the eggs of bladderworms are known to disperse great distances, from meters to over 50 km (Gemmell and Johnstone 1976, Gemmell et al. 1987, Torgerson et al. 1995). With these biological characteristics, it is unsurprising that bladderworm infection was not strongly correlated with any cover types. The weak positive correlation between mixed forest and number of infected deer could be related to the definitive host habitat. In Duluth, there are four definitive hosts most likely associated with bladderworm infection

in deer, coyotes (*Canis latrans*), red foxes (*Vulpes vulpes*), gray foxes (*Urocyon cinereoargenteus*), and domestic dogs (*Canis lupus familiaris*). Although wolves (*Canis lupus*) are the most common definitive host (Jones and Pybus 2001), it is unlikely that wolves come far within city limits. If the four urban canids above are using mixed forest habitats for cover or foraging within the city, these habitats could carry a higher risk of bladderworm infection for deer.

The current study has outlined a potential methodology and baseline results for monitoring liver fluke and bladderworm infection and coinfection in an urban deer population. We recommend continued monitoring of these populations in order to better understand giant liver fluke and bladderworm disease dynamics on the urban landscape. The additions of DHA deer density estimates, deer aging techniques, and GPS collaring of urban deer would be valuable for a comprehensive understanding of these disease systems.

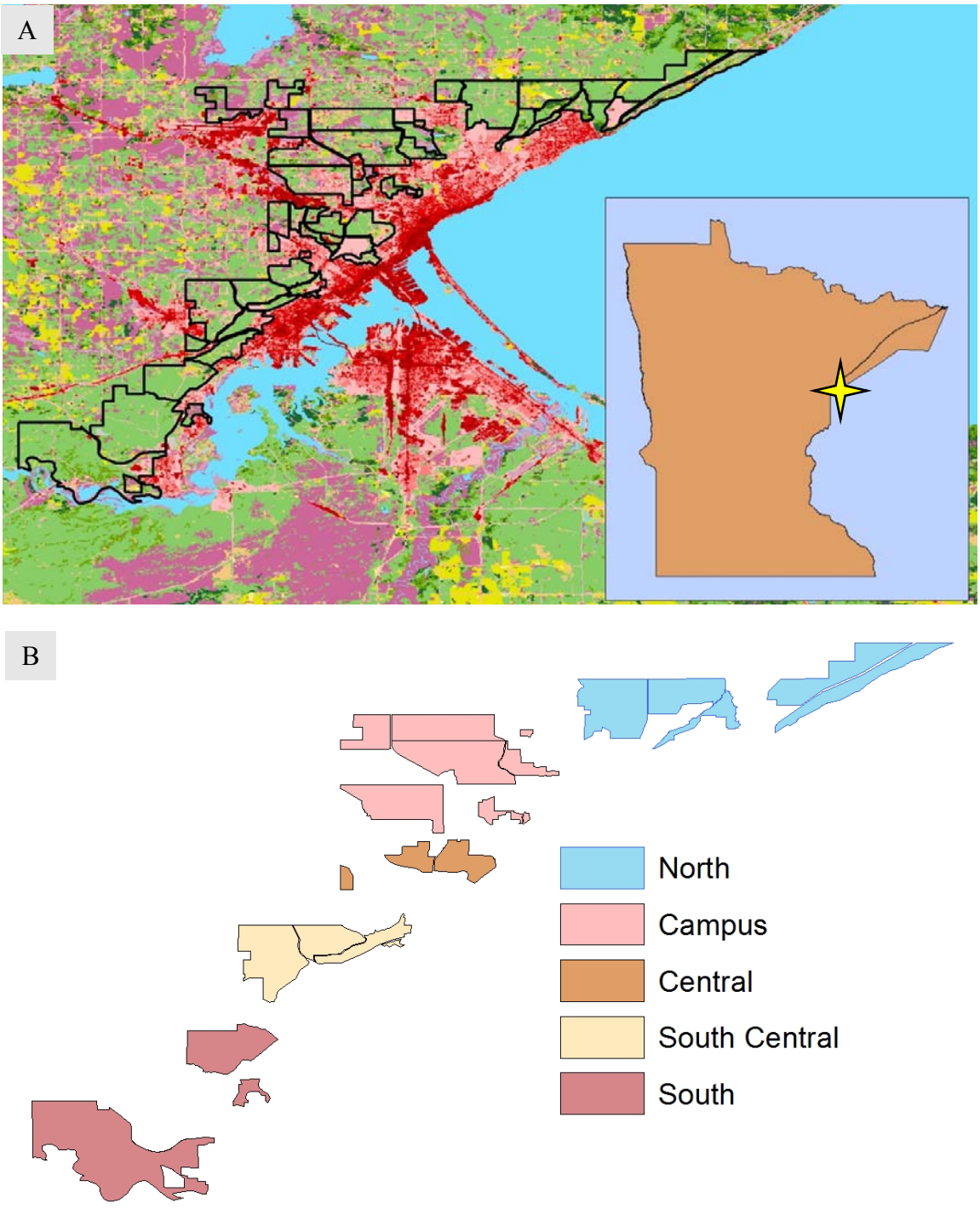

Figure 1. (A) Black lines outline the various deer hunting areas (DHAs) in the city of Duluth, Minnesota, USA overlaid on the National Land Cover Dataset (NLCD) 2011 raster. Inset shows Minnesota with a star indicating Duluth. (B) DHA subregions from which livers were received in 2015.

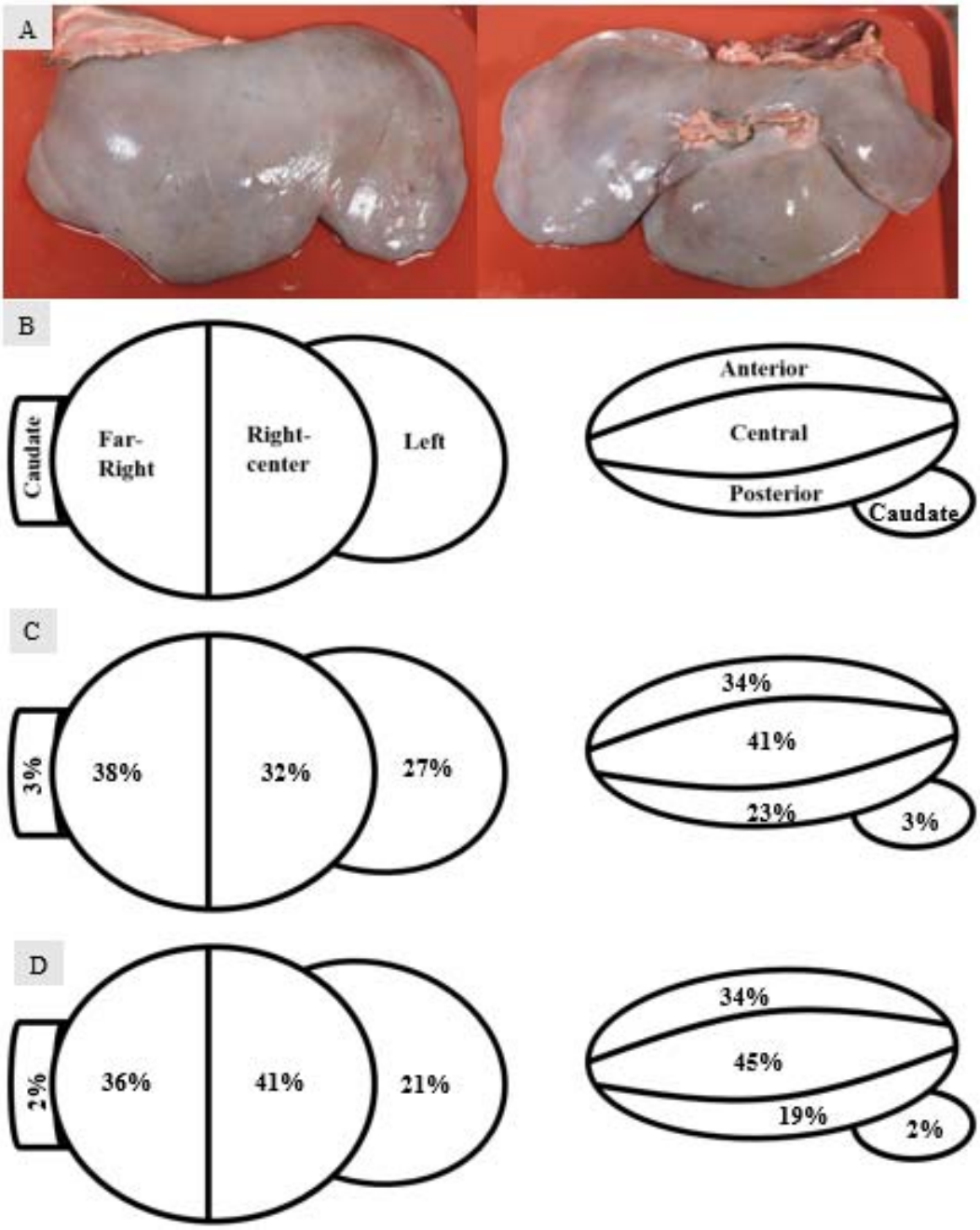

Figure 2. (A) Anterior (left) and Posterior (right) surfaces of a liver. (B) Diagrams of a liver's anterior (left) and lateral (right) surfaces with regions noted. (C) Percent of capsules found in each liver region (N = 77). (D) Percent of flukes found in each liver region (N = 150).

Table 1. DHA subregions with various measures of liver fluke and bladderworm (*Taenia*) infection.

District	Number of deer	Mean liver weight (g)	Fluke infected deer	Fluke prevalence	Number of flukes	Flukes per deer	Fluke mass (g)	Fluke mass per deer (g)	*Taenia* infected deer	*Taenia* prevalence	Number of *Taenia*	*Taenia* cysts per deer
North	11	999	5	0.45	9	1.80	12.23	2.45	2	0.2	3	1.5
Campus	13	1058	8	0.62	26	3.25	77.77	9.72	3	0.2	9	3.0
Central	6	1160	3	0.50	16	5.33	9.89	3.30	2	0.3	10	5.0
South Central	15	1177	7	0.47	41	5.86	70.08	10.01	2	0.1	6	3.0
South	8	1114	3	0.38	12	4.00	33.90	11.30	4	0.5	8	2.0

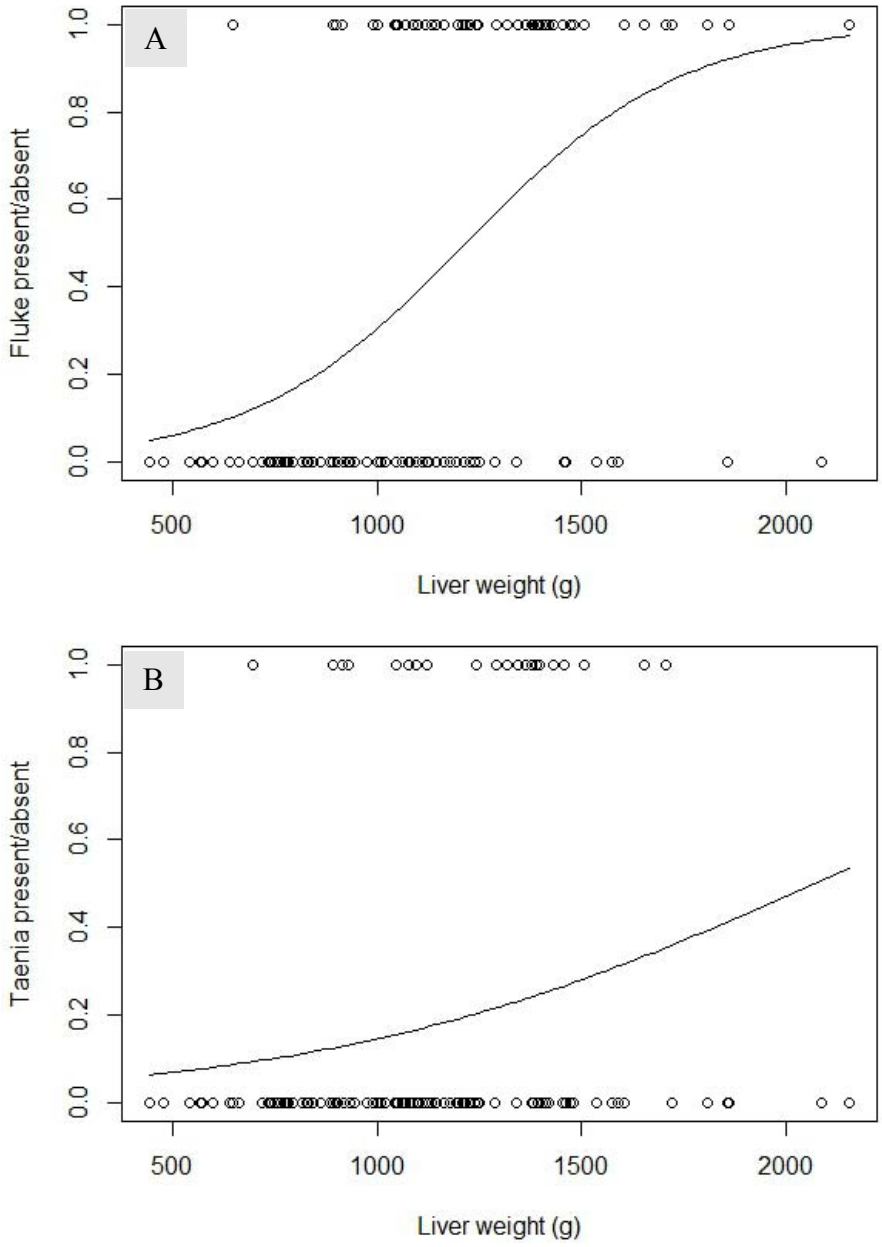

Figure 3. (A) Generalized linear model of liver weight (g) against fluke presence / absence. Liver weight (X_{LF}) was a significant explanatory variable of fluke presence ($p << 0.0001$) using the logistic model logit (π_{LF}) = $-4.63 + 0.0038X_{LF}$. Liver weight significantly decreased model deviance ($p << 0.0001$) and modelled data was not significantly different from the observed data (Hosmer-Lemeshow goodness of fit test, $\chi^2 = 13.61$, df = 8, $p = 0.09$). (B) Generalized linear model of liver weight (g) against bladderworm (*Taenia*) presence / absence. Liver weight (X_B) was a significant explanatory variable of bladderworm presence ($p = 0.02$) using the logistic model logit (π_B) = $-3.41 + 0.0016X_B$. Liver weight significantly decreased model deviance ($p = 0.02$) and modelled data was not significantly different from the observed data (Hosmer-Lemeshow goodness of fit test, $\chi^2 = 12.37$, df = 8, $p = 0.14$).

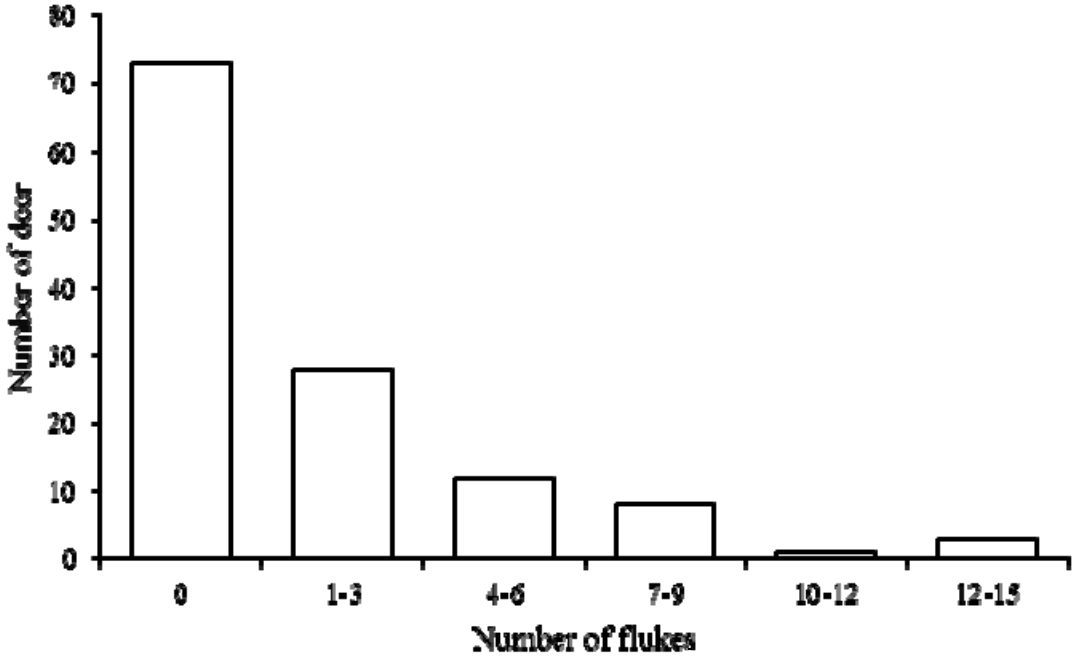

Figure 4. Number of flukes within deer livers. 77% of deer had between 1 and 6 flukes. However, 52% of the sampled fluke population (120 out of 229 flukes) were carried by only 10% of the harvested deer population.

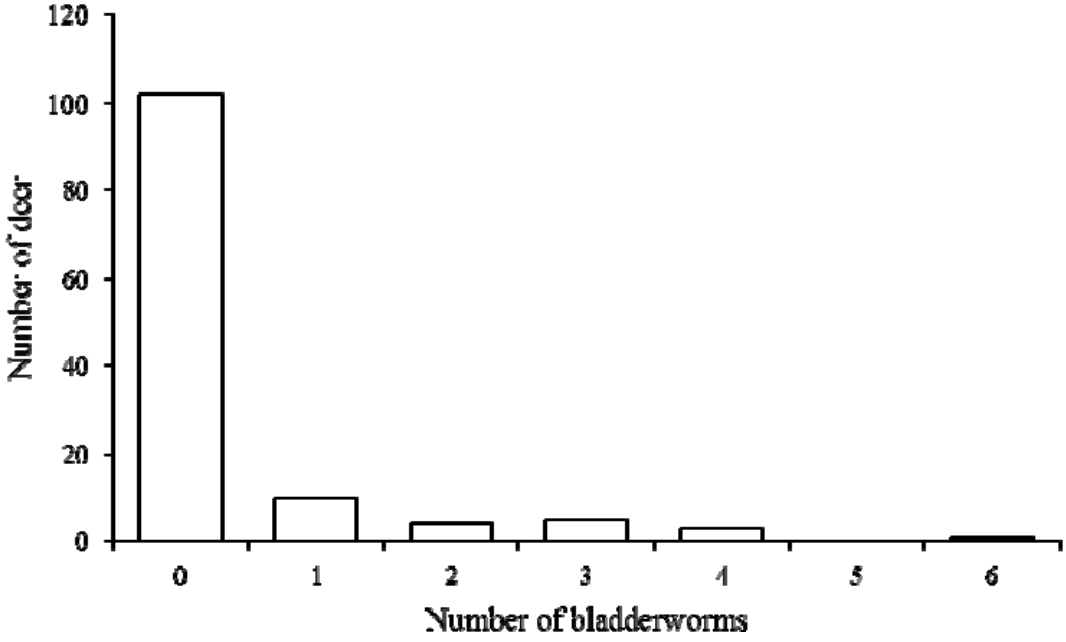

Figure 5. Number of bladderworms within deer livers. 35% of bladderworms (N = 18) were carried by 17% of infected deer or 3% of the harvested deer population.

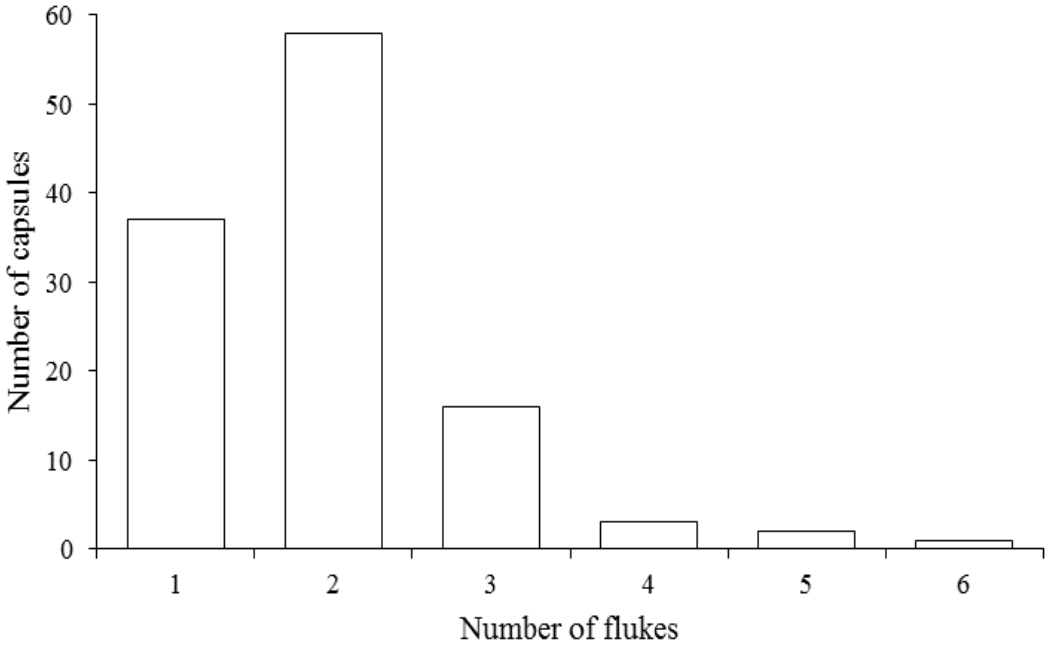

Figure 6. Number of flukes within capsules. Flukes in Duluth, Minnesota demonstrate a pairing tendency found in other populations (Foreyt et al. 1977).

Table 2. Linear regression summaries.

Variables (x, y)	Regression equation	R^2 value	P value
Liver weight v. Number of flukes	y = 0.0043x – 2.953	0.21	< 0.0001
Liver weight v. Number of capsules	y = 0.002x – 1.3154	0.19	< 0.0001
Liver weight v. Number of bladderworms	y = 0.0006x – 0.3042	0.04	0.02
Liver weight v. Fluke length	y = 0.0187x + 39.867	0.12	0.01
Liver weight v. Fluke width	y = 0.008x + 13.137	0.11	0.02
Liver weight v. Total fluke mass	y = 0.0019x – 0.6252	0.19	0.004
Liver weight v. Mean fluke mass	y = 0.0172x – 12.05	0.16	0.002
Liver weight v. Liver volume	y = 0.8765 + 24.054	0.94	< 0.0001
Fluke length v. Fluke mass	y = 6.3102x + 53.254	0.35	< 0.0001
Fluke width v. Fluke mass	y = 3.8844x + 17.612	0.47	< 0.0001
Developed open space v. Liver weight	y = 1328.3x + 931	0.61	0.12
Evergreen forest v. Liver weight	y = -4709.9x + 1164.5	0.97	0.002
Deciduous forest v. Fluke prevalence	y = -0.778x + 0.9253	0.78	0.05
Low to moderate development v. Fluke prevalence	y = 3.6517x + 0.2845	0.94	0.007
Shrub/scrub v. Fluke prevalence	y = 2.7913x + 0.2961	0.96	0.003
Open water v. Fluke mass	y = -96.01x + 41.62	0.003	0.93
Mixed forest v. Number of bladderworm infected deer	y = 27.744x + 1.6735	0.57	0.14
Evergreen forest v. Prevalence of Coinfection	y = 5.5523x + 0.1199	0.75	0.06

Table 3. Proportions of NLCD 2011 land cover types in each DHA subregion. Wet cover consists of woody wetlands, emergent herbaceous wetlands and open water.

District	Wet Cover Types	Developed Open Space	Developed Low to Moderate	Deciduous Forest	Evergreen Forest	Mixed Forest	Shrub/ Scrub	Hay/ Pasture	*Woody Wetlands*	*Emergent Herbaceous Wetlands*	*Open Water*
North	0.05	0.07	0.05	0.62	0.03	0.04	0.05	0.06	*0.04*	*0.01*	*0.00*
Campus	0.20	0.14	0.09	0.40	0.02	0.03	0.12	0.01	*0.14*	*0.05*	*0.00*
Central	0.06	0.16	0.07	0.62	0.00	0.02	0.07	0.01	*0.05*	*0.01*	*0.00*
South Central	0.12	0.18	0.05	0.57	0.00	0.01	0.07	0.00	*0.08*	*0.03*	*0.00*
South	0.12	0.09	0.03	0.64	0.01	0.07	0.03	0.01	*0.06*	*0.01*	*0.04*

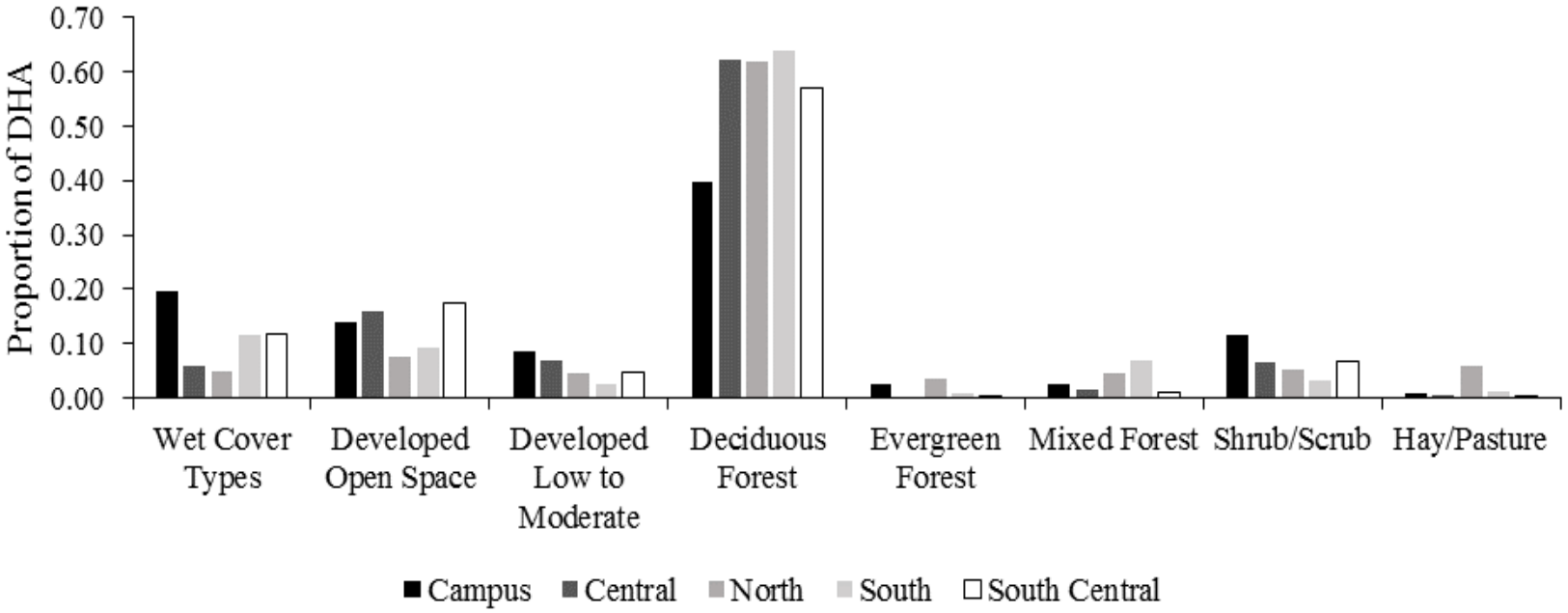

Figure 7. Cover types and the proportion of each DHA subregion they contribute.

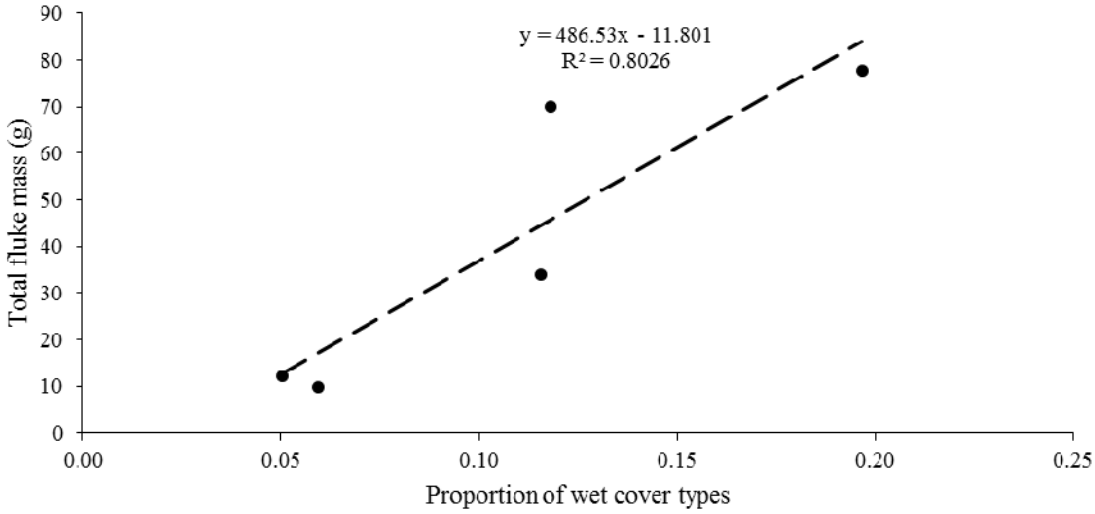

Figure 8. Correlation between proportion of wet cover types and total fluke mass (p = 0.04).

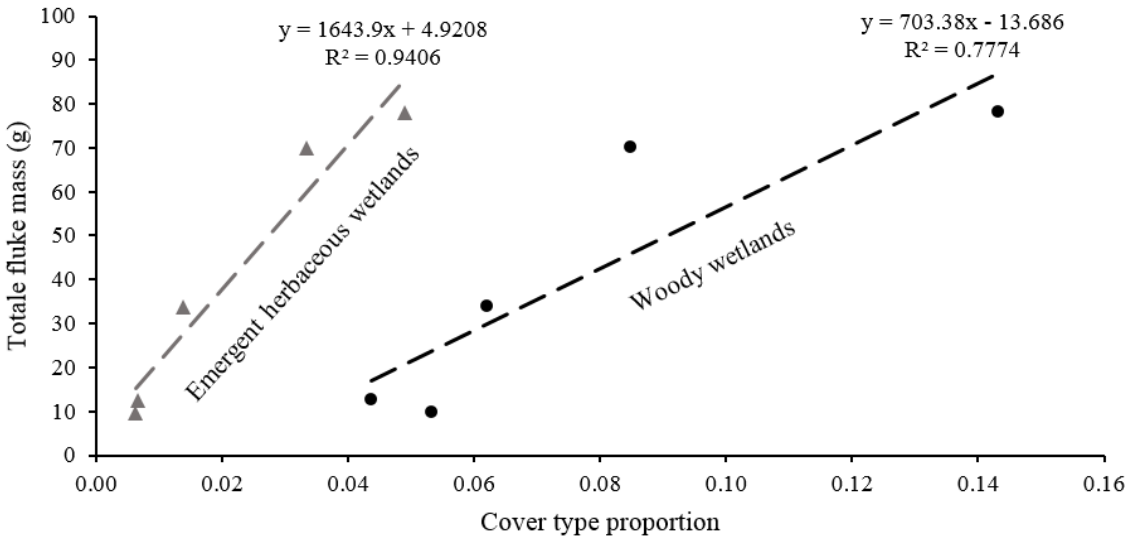

Figure 9. Correlation between proportions of woody wetlands (p = 0.05) and emergent herbaceous wetlands (p = 0.006) and total fluke mass.

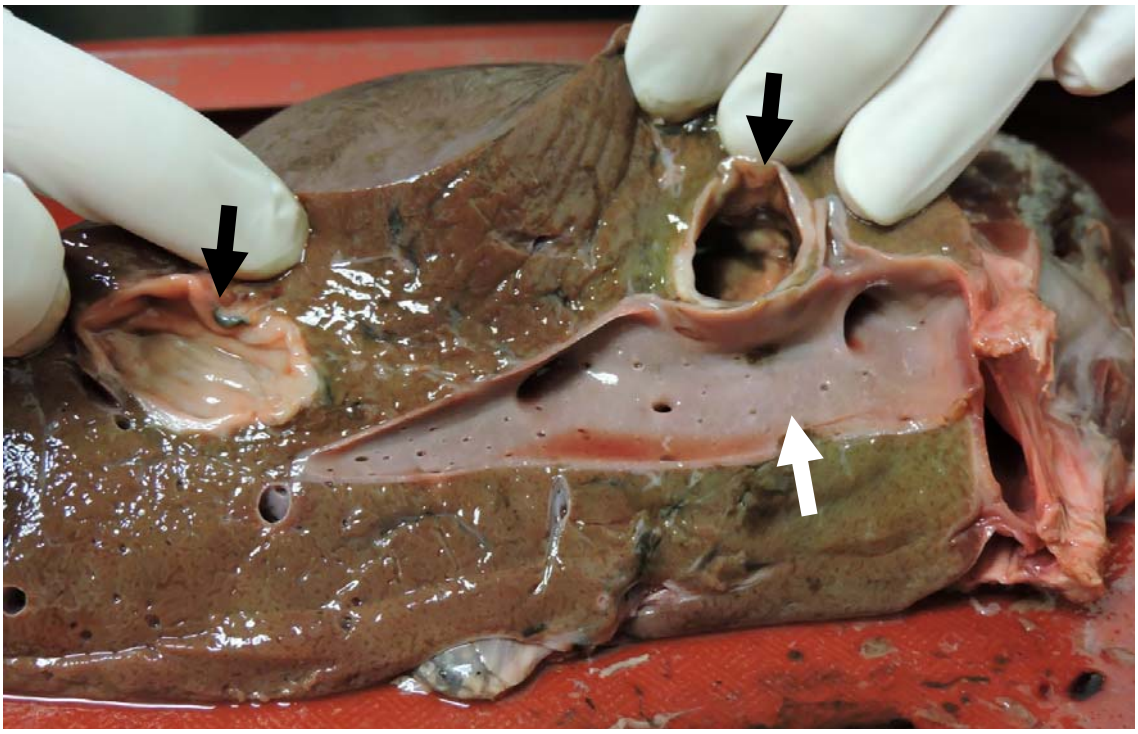

Figure 10. Two fluke capsules (dark arrows) in close proximity to a major hepatic blood vessel (light arrow).

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